

RETENTION OF N-15 LABELLED UREA IN A RADIATA PINE ECOSYSTEM.  
-- EFFECT OF SPLIT APPLICATIONS

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For my father

and in memory of

Rev. R.F.D. Thomas

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## ABSTRACT

The efficiency of fertilizer nitrogen uptake and retention within the ecosystem were studied in a 2-year-old radiata pine stand. This was a third rotation site on coastal sand dunes. Applications of 90 g N/tree (150 kg N/ha), as either Single, 3-Split or 9-Split dressings, were applied as urea solution, labelled with 2.69 atom % N-15. A Control and three seasonal treatments (30 g N/tree) were also included. The trees were in the centre of 7 m<sup>2</sup> root isolated plots.

The experiment ran for a period of 17 months during which time N-15 uptake was monitored by foliar analysis. Steady levels were reached 6 months after the Single application. This indicated that soil processes acted on the N-15 pool to achieve an equilibrium with soil-N.

The final distribution of fertilizer nitrogen within the ecosystem was determined by a complete tree harvest (including roots) and soil sampling to 90 cm. There was a 25% increase in above ground biomass formed in the year after fertilization with 90 g N/tree. The below ground response was more marked, with an 80% increase in fine (<2 mm) roots. The only detectable response to 30 g N/tree was below ground.

Irrespective of the rate, the season or the splitting of the application, the tree's uptake of fertilizer nitrogen was the same (mean = 21.3%). However the retention within the soil varied from 40% with a Single application to 70% for the 9-Split application. The total ecosystem retention varied from 60% (Single) to 90% (9-Split) excluding volatilization (<2%) and uptake by surrounding trees (<3%). The retention for the seasonal treatments was similar to the split treatments.

There was a positive relationship between tree uptake of fertilizer nitrogen and initial sink size. Other major factors determining the range of tree uptakes (15-32%) were leaching and immobilization. The high soil retention of fertilizer nitrogen at the lower application rate, and for split applications, suggests immobilization is a dominant process. Leaching was most prominent for the Single 90 g N application. The extent of this loss may have precluded further uptake by the tree.

Although total retention was increased with split applications, there was no immediate benefit to the tree. Given that this additional nitrogen is a very minor proportion of the total in the soil, the probability of additional responses seems unlikely.

Split applications did not increase tree uptake of nitrogen. Indeed, in the absence of leaching a single application may be utilised more efficiently.

## CHAPTER 1

## GENERAL INTRODUCTION

*New distant scenes of endless science rise!  
So pleas'd at first the tow'ring Alps we try,  
Mount o'er the vales, and seem to tread the sky,  
Th' eternal snows appear already past,  
And the first clouds and mountains seem the last;*  
Pope (1711)

The importance of nutrients in forest ecosystems has long been recognised (see Tamm (1979) for historical review). However it is only in the last three decades that nutrient cycles have been intensively studied. This development together with an expansion of plantation forestry has led to an interest in fertilizers as a silvicultural tool.

Forest produce has traditionally been obtained from natural forests with management often aiming to regenerate stands by natural means. Now many countries have established plantation resources to redress the balance of past deforestation, to alleviate demand on remaining natural resources, to ensure timber supplies and to meet a variety of social and environmental objectives. Exotic species have often been introduced, notably conifers from north and central America and species of *Eucalyptus* from Australia.

Plantations will become increasingly important in meeting the world's demand for wood (Campbell 1980). They have usually been established on marginal or non productive agricultural land. Some of these plantations have been highly productive, for example, radiata pine (*Pinus radiata* D. Don) on the volcanic soils of the central North Island of New Zealand. However, there are many where nutritional problems occur, and a response to one or more added nutrients is likely (Nambiar 1984a).

Initially fertilizers were used to correct chronic deficiencies as in the phosphorus deficient clays north of Auckland, New Zealand (Weston 1956). Although "starter" and "corrective" fertilization are still widespread (Flinn 1984), it is now common to use fertilizers to achieve a variety of management goals (Hunter 1984) including the promotion of growth in already highly productive stands (Woollons and Will 1975).

Plantations are in a dynamic state with regard to biomass accumulation and nutrient use. Attiwill (1979) proposes three defineable stages of growth : growth of living biomass, heartwood formation and stage of maintenance. This sequence regulates net primary production and the accumulation of biomass. Similarly Miller (1981) proposes three nutritional stages in the life of a forest stand. During Stage I the demand for nutrients is high as canopies and root

systems expand to occupy the site. Once canopy closure occurs and a nutrient cycle develops through litterfall, Stage II is reached and the call on soil resources lessens. The reuse of nutrients within the tree also becomes important (Miller 1984). Stage III occurs when accumulation of litter immobilizes much of the potentially available nitrogen. Fertilizers may usefully be applied to hasten the initiation of Stage II. In South Australia on poor podzolised sands this has been recognised during the establishment phase of radiata pine by adopting an intensive fertilization and weed control regime (Woods 1976). A response to nitrogen may also be anticipated in Stage III, but this may be more applicable to northern hemisphere boreal forests where climate induces longer rotations and a lower turnover of nitrogen.

A further stage at which fertilizer applications may be beneficial is after perturbations in the above cycle, such as thinning. This can be viewed as a temporary reversion to Stage I. Responses to nitrogen after thinning have been explained in this manner (Hunter 1982, Crane 1985).

Although nitrogen is the most abundant element in the atmosphere it is often the most limiting to forest growth. The importance of a balanced nutrient supply has been stressed by Ingestad (1979) and others (see Mead 1984). However provided other nutrients are not limiting there is great scope for increasing forest productivity by supplying nitrogen. This may be achieved by applying nitrogenous fertilizers or by utilising nitrogen fixing organisms (Davey and Wollum 1984).

Pritchett (1979) reports that large areas of pine and spruce in Scandinavia are fertilized each year with nitrogen as are extensive forests of douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) in the American Pacific North West. Will (1985) reported a usage of 1900 tonnes elemental nitrogen in New Zealand forests in 1980. This had dropped to 1000 tonnes by 1983 and is likely to have declined further (Will pers. comm.), due to a tighter economic environment.

In New Zealand there are 1.01 million hectares of radiata pine (Lavery 1986). The 45,000 hectares of sand dune plantations are inherently low in nitrogen (c.f. Hunter and Hoy 1983) as are plantations in Westland. These may be termed nitrogen deficient sites. However, at least half of the plantation resource might respond to nitrogen at some stage of stand development (Will pers. comm.). The present study looks at some aspects of the nitrogen nutrition of radiata pine on a coastal sand site.

The efficiency of nitrogen fertilizer use may be defined as the percentage recovery of nitrogen fertilizer by a crop (Parr 1973). For agricultural crops this is usually in the range 30-80% (Black 1967). Studies to date in forestry rarely show an efficiency greater than 20% (see Ballard 1984, Melin *et al.* 1983), and the total recovery within the studied ecosystem is also usually low (Ballard 1980). This low efficiency of fertilization is disturbing both from an economic viewpoint and for the possible adverse environmental consequences of

nitrogen leakage.

The research reported on in this thesis looks at one possible method for improving the uptake of nitrogen fertilizer, namely split applications. This is defined as repeated doses within the growing season rather than in successive growing seasons, termed multiple applications, as advocated by some workers (Woollons and Will 1975, Weetmen and Fournier 1984).

It is postulated that smaller more frequent applications of nitrogen fertilizer can be better utilised by the plant as this will be similar to their supply of native soil nitrogen. This approach has been successfully adopted with young tree seedlings in a hydroponic environment (Ingestad 1982). With an exponential increase in growth there is a concomitant uptake of nutrients giving constant internal nutrient concentrations. Ingestad (1974, 1977) believes fertilization programmes should be optimized to meet plant requirements and counter the risk of environmental damage. He contends that it is possible to achieve nearly one hundred per cent efficiency in applying nitrogen to plants. In a field experiment designed to test this hypothesis nutrients were supplied almost daily as a very dilute solution to young scots pine (*Pinus sylvestris* L.) for several growing seasons (Aronsson and Elowson 1980). Some nitrogen recovery results from this trial were presented by Ingestad *et al.* (1981). Of the 670 kg N/ha supplied, 76% was recovered within the soil and vegetation. The recovery within the tree biomass was 20% of that applied; the same as many studies with conventional applications of fertilizer. No comparative data were presented for the solid fertilizer treatment where multiple applications were adopted. Without a more accurate balance sheet for the applied nitrogen and a comparison with other treatments, it is not possible to say whether this irrigation and fertilizer treatment gave a superior tree uptake and ecosystem recovery of applied nitrogen. Although high tree recovery is one aim of Ingestad's work it should be appreciated that the major objective is to gradually saturate the system with nutrients. This increases the nutrient flux density, initially by fertilizer additions but later by the feed back of fertilization on the mineralization rate. A steady state is visualised when mineralization is able to provide sufficient nitrogen for optimum uptake and fertilization is only required to balance nitrogen removed in stem harvests (Ingestad and Agren 1984).

Split applications are a possible move towards the ideal of a high tree utilisation and ecosystem recovery and have achieved success in some agricultural systems (Mengel and Kirkby 1982. p.315). Hamid (1972) utilised split applications of nitrogen on wheat and found increased efficiency over a single application. There were however differences between ammonium and nitrate sources with the former, not being beneficial beyond two split doses. Nagarajah *et al.* (1975), working with rice, conclude that factors such as soil type, variety of rice and season will determine whether split applications are superior.

Foresters have been slow to learn from agricultural and

horticultural practices, which is, perhaps, surprising as we may be termed tree farmers (Davies 1979). Lynch (1974) considered that in designing forest fertilization experiments, the results from agricultural experience should be heeded and treatments approximating those in agriculture tried. The applicability of results from annual crops to trees is debatable and concepts developed with small seedlings (Ingestad 1982) are more difficult to visualise in large trees (Cromer *et al.* 1985).

There is, however, some support for the superiority of split applications in the literature on forest trees. The recovery of fertilizer within trees tends to decrease at higher application rates (Tamm 1982). The splitting of the amount to be applied may thus improve uptake, and some workers have assumed this when deciding how to apply fertilizer in experiments (Mead *et al.* 1984). However, there is very little direct evidence for this assumption, the only positive trial being in young loblolly pine (*Pinus taeda* L.). By applying 224 kg N/ha in two instalments rather than one, Baker *et al.* (1974) increased recovery from 3 to 7%. The authors concluded that the efficiency of nitrogen utilization can be improved by applying lower rates of nitrogen throughout the growing season. One should perhaps be sceptical though; the study relied on the difference between nitrogen in fertilized and unfertilized plots, the total amount recovered within the ecosystem was only 20-30%, and there was a serious weed problem.

A number of trials have been conducted in Sweden with split applications. Eriksson (1982) showed that on the basis of foliar nitrogen analysis a positive growth effect from splitting 150 kg N/ha three or six times could be expected, but after five growing seasons no significant differences in basal area growth response were found. In a series of follow up trials Pettersson (1984) similarly found no significant difference between treatments in terms of volume increment. In the only split application study to budget for the applied nitrogen using N-15, Melin *et al.* (1983) found no significant difference in fertilizer recovery between a single 100 kg N/ha and 2 x 50 kg N/ha applications applied to scots pine. It should be noted that this Swedish work was generally in stands 100 years old or greater.

In the only other known trial with split applications, Szczesny (1977) used foliar nitrogen to assess their effectiveness in 30-year-old scots pine in Poland. He found two or four applications superior to a single dose.

In the case of multiple applications, cited earlier, it has been shown by Tustin and Mead (1973) that no additional cost is incurred over fertilizing in one year. With split applications the benefit of not carrying the investment cost for as long does not apply. The extra cost of applying and supervising several applications within the year must be met by improved fertilizer efficiency. In practice this would mean using less fertilizer to achieve the same response. The data in Table 1.1 compares the cost of a Single and a 3-Split application.

Table 1.1 Cost of applying 150 kg N/ha as urea in a Single or an equal 3-Split application.

Costs*	Single	3-Split
\$/ha		
Urea @ \$420/t	137	3 x 45
Helicopter	20	3 x 15
Total	157	182

\*: N.Z.F.S. (pers.comm.) Jan. 1987.

The opportunity cost of using a 3-Split application is \$25/ha. The improvement in efficiency of fertilizer uptake required to meet this cost may be calculated. A \$25 saving can be made if 27 kg N/ha less fertilizer is required, i.e. if an application rate of 123 kg N/ha as opposed to 150 kg N/ha is used. If a 20% fertilizer efficiency is assumed for a single 150 kg N/ha application what efficiency is required for a 3-Split application totalling only 123 kg N/ha? Clearly to achieve 30 kg N/ha in trees ( $20\% \times 150 \text{ kg N/ha}$ ), the uptake from the 3-Split must be 24% ( $30 \times 100 \div 123 \text{ kg N/ha}$ ). This calculation is dependent on the relative cost of fertilizer and flying. If urea becomes relatively more expensive, then the increased efficiency required from split applications decreases.

The evidence in the literature for the superiority of split applications is inconclusive. Foliar analysis suggests positive benefits but fertilizer recovery and growth responses generally do not. The economic calculation shows that increasing the uptake of fertilizer by only a few percent justifies the use of split applications. If retention of fertilizer within the ecosystem is increased, the environmental benefits and possible site improvement should also be used to offset the additional costs of split applications.

There has been no rigorous test of nitrogen recovery in a young pine ecosystem using split applications. The experiment described in the following chapter was thus initiated to test whether split applications affected the uptake and distribution of N-15 labelled urea in a young radiata pine stand.

Urea was used, as with the exception of Scandinavia this fertilizer is the major nitrogen source for forest fertilization. In New Zealand its use may increase with the recent completion of the ammonia-urea plant at Kapuni.

Because only a small improvement in fertilizer efficiency is required to make split applications an economic proposition, a precise means of measuring fertilizer recovery was required. For this and other reasons discussed later the use of urea labelled with the heavy isotope of nitrogen, N-15, was particularly appropriate.

## CHAPTER 2

## THE EXPERIMENT

In 1983 an experiment was laid out in Bottle Lake Forest, Christchurch, New Zealand (Lat.43°S, Long.173°E), to study the uptake and subsequent distribution of nitrogen following split applications of N-15 enriched urea.

### 2.1 Site

This forest is located on an area of coastal sand dunes, afforested from 1912 onwards to control the drift of sand onto agricultural land. A variety of tree species were tried, but early on the superiority of radiata pine was evident, and this is now the major species.

A third rotation site of two-year-old radiata pine was chosen (Figure 2.1). This was predominately flat with sparse grasses and patches of bracken and blackberry. The water table was never within one metre of the surface during the study. During the course of the experiment, this stand would be in a dynamic phase as regards nutrient and biomass accumulation. Furthermore, it is a relatively simple, uniform system with a recent undifferentiated soil, no litterfall and trees small enough for relatively easy sampling and biomass determinations.



Figure 2.1 The site in May 1983 at the beginning of the experiment.



## 2.2 Stand History

Compartment 29 was first planted with radiata pine in 1933. This was windblown in 1955 and replanted. In August 1975, this stand along with half of the forest was windblown in the famous Canterbury gale described by Wilson (1976). After clearing and burning the area was planted in August 1981, with 1.5-year-old radiata pine at 2250 stems/ha. Planting stock for Bottle Lake is raised at Halkett Nursery, near Christchurch, from seed collected in a stand adjacent to the study area. The original planting stock for Bottle Lake probably came from Ashburton, 60 km south of Christchurch.

## 2.3 Soils

Soil characteristics determined during the study are given in Table 2.1a. Details of their measurement are given in Chapters 5 and 7. Additional soil data for this Kairaki sand (Table 2.1b) were obtained from Dr J.A. Adams, Lincoln College. They are compatible with the 0-10 cm horizon given in Table 2.1a as the samples came from a site near the experiment.

Table 2.1a Soil characteristics determined on the plots.

Depth (cm)	Bulk density (g/cm <sup>3</sup> )	Total N (%)	Organic matter (%)	pH	C (%)	C:N
0-10	1.24	0.064	3.71	4.8	2.15	34:1
10-30	1.46	0.019	1.73	5.0	1.00	53:1
30-50	1.49	0.011	0.98	-	0.57	52:1
50-70	1.47	0.008	0.82	-	0.47	55:1
70-90	1.48	0.006	0.74	-	0.43	65:1

Table 2.1b Additional soil data for Bottle Lake Forest.

Sand (%)	98
Silt (%)	2
Clay (%)	0
Total P (ppm)	280
Bray 2P (ppm)	16.3
Exchangeable Ca (me/100g)	0.93
Exchangeable Mg (me/100g)	0.76
Exchangeable K (me/100g)	0.22
C.E.C. (me/100g)	5.8
Base saturation (%)	33

## 2.4 Experimental Preparation

The experimental design utilised single tree plots. Twenty two trees were selected in a 0.25 ha area at the corner of the compartment. They were chosen so that between tree and plot variability was at a minimum according to the following criteria:

Tree height.

Tree diameter.

Absence of double leader and other malformations.

Proximity to old stumps.

Absence of significant vegetation such as bracken.

Distance from small dunes.

Initial investigations showed that roots from 2-year-old trees already extended at least two metres from the stem. To prevent cross feeding between plots, each tree was isolated by trenching at 1.5 m radius to a depth of 75 cm, inserting polythene sheeting and backfilling (Figure 2.2). To further ensure homogeneity between plots any logging slash was removed and vegetation clipped off. Remerging weeds were controlled throughout the experiment by applying glyphosphate (Roundup) monthly, using a weedwiper.



Figure 2.2 Plot preparation.

## 2.5 Experimental Design

Experimental preparation was completed by April 1983. Plots were randomly allocated to the treatments shown in Table 2.2.

Table 2.2 Experimental design

Treatment name	Grams N applied per tree	kg N/ha equivalent	Replication	Fertilizing dates (1983)
Control	0	0	4	-
Single	90	150	4	August
3-Split	30 x 3	150	4	May, August, December
9-Split	10 x 9	150	4	monthly, May-December
Autumn	30	50	2	May (autumn)
Spring	30	50	2	August (early spring)
Summer	30	50	2	December (early summer)

The design allows the data for various parameters (e.g. height, biomass, fertilizer recovery) to be statistically analysed for four main treatment responses:

The response to split applications.

The response to 30 grams nitrogen in different seasons.

The response to 30 grams nitrogen per tree.

The response to 90 grams nitrogen per tree.

These maybe formalised as two null hypotheses:

$H_0$ : Control = Single = 3-Split = 9-Split

$H_0$ : Control = Autumn = Spring = Summer

The lower replication for the seasonal treatments was a consequence of fertilizer cost and work required. These treatments should thus be viewed as subsidiary to the main experiment. Their main role was to ascertain if any superiority of the split applications was due to smaller, more frequent doses *per se* or to seasonal differences in uptake.

Overall treatment differences were tested using analyses of variance (ANOVA). Where initial tree size was thought to be a major factor, analyses of co-variance (ANCOVA) were used to remove this effect. This was appropriate for the main treatments, individually ( $n=4$ ) and the seasonal treatments combined ( $n=6$ ). Individual treatment differences were tested for using single degree of freedom contrasts. Where this was not possible, because of unequal replication, Duncan's multiple range test was used.

## 2.6 Fertilizer Applications

The first application was made on 2 May 1983. All applications used urea (46% nitrogen) enriched at 2.69 atom % N-15. They were applied in solution to ensure an even spread and to minimize volatilization losses (Worsnop and Will 1980, Volk 1970). At each application the non-fertilized plots also received the same amount of water, i.e. fifteen litres which was equivalent to 2.4 mm rainfall. A margin (approximately 10 cm wide) was left unfertilized around the plot edge to prevent fertilizer running down the plastic (Figure 2.3).



Figure 2.3 Fertilizer application on 30 May 1983.

The final application was on December 13 1983. The trial then ran for a further 10 months until 9 October 1984. The response period after application thus varied from 10-17 months. As most growth occurred from November to March (Chapter 3), it was felt that all treatments essentially had a response period of one growing season.

In May 1983, at the start of the experiment, the mean height of experimental trees was 1.60 m and the diameter at the base of the stem 40 mm. The nutritional status of the trees on 2 May 1983 is given in Table 2.3. Details of analytical methods and interpretation are given in Chapter 4. Although May is not the usual time for foliage sampling in New Zealand (Mead and Will 1976, Will 1985) it appears that all nutrients are in adequate supply for the growth of radiata pine, although responses to nitrogen have sometimes been observed in stands with foliar nitrogen levels in excess of those reported here (Mead and Gadgil 1978).

Table 2.3 Foliar nutritional status of 2-year-old radiata pine at Bottle Lake in May 1983.

% oven dry weight								
N	P	K	Ca	Mg	Si	Cl	S	Al
1.58*	0.16	0.88	0.28	0.13	0.04	0.19	0.12	0.04

\*: The summer minimum for control trees in February was 1.47 % N.

## 2.7 Climate

Two standard rain gauges were located at either end of the site, approximately 50 m apart. Rainfall was recorded daily throughout the experiment and is summarised on a weekly basis in Figure 2.4. Air temperature was monitored continuously using a thermohygrograph in a Stevenson's screen one metre above ground level. Daily maximum and minimum averaged for the week are also shown in Figure 2.4.

The longest periods without rain were from 4-21 August 1983 just prior to the Single fertilizer application, and from 9-26 April 1984. The highest recorded temperature was 33°C on 24 February 1984, the lowest -5°C on June 25 1984. Rainfall data prior to, and following each fertilizer application are given in Table 2.4.

Table 2.4 Rainfall prior to and following each fertilizer application.

Application date (1983)	Treatments receiving fertilizer	Daily rainfall										Rainfall within 1 week
		prior				(mm)	after					
		4	3	2	1	F*	1	2	3	4		
2 May	Autumn, 3-Split 9-Split	28	0	0	0	0	0	0	0	0	7	
30 May	9-Split	0	0	0	0	11	0	0	0	0	11	
27 June	9-Split	0	0	0	0	0	0	1	0	7	31	
26 July	9-Split	0	9	0	0	0	0	0	0	0	0	
22 August	Spring, 3-Split 9-Split, Single	0	0	0	0	2	1	15	0	0	18	
20 September	9-Split	0	0	0	0	0	0	0	0	0	36	
19 October	9-Split	0	0	0	0	0	0	0	0	1	3	
15 November	9-Split	0	0	0	2	0	0	0	0	0	0	
13 December	Summer, 3-Split 9-Split	0	0	12	3	3	26	2	18	0	63	

F\* Day of application, rainfall does not include 2 mm equivalent used to apply urea.

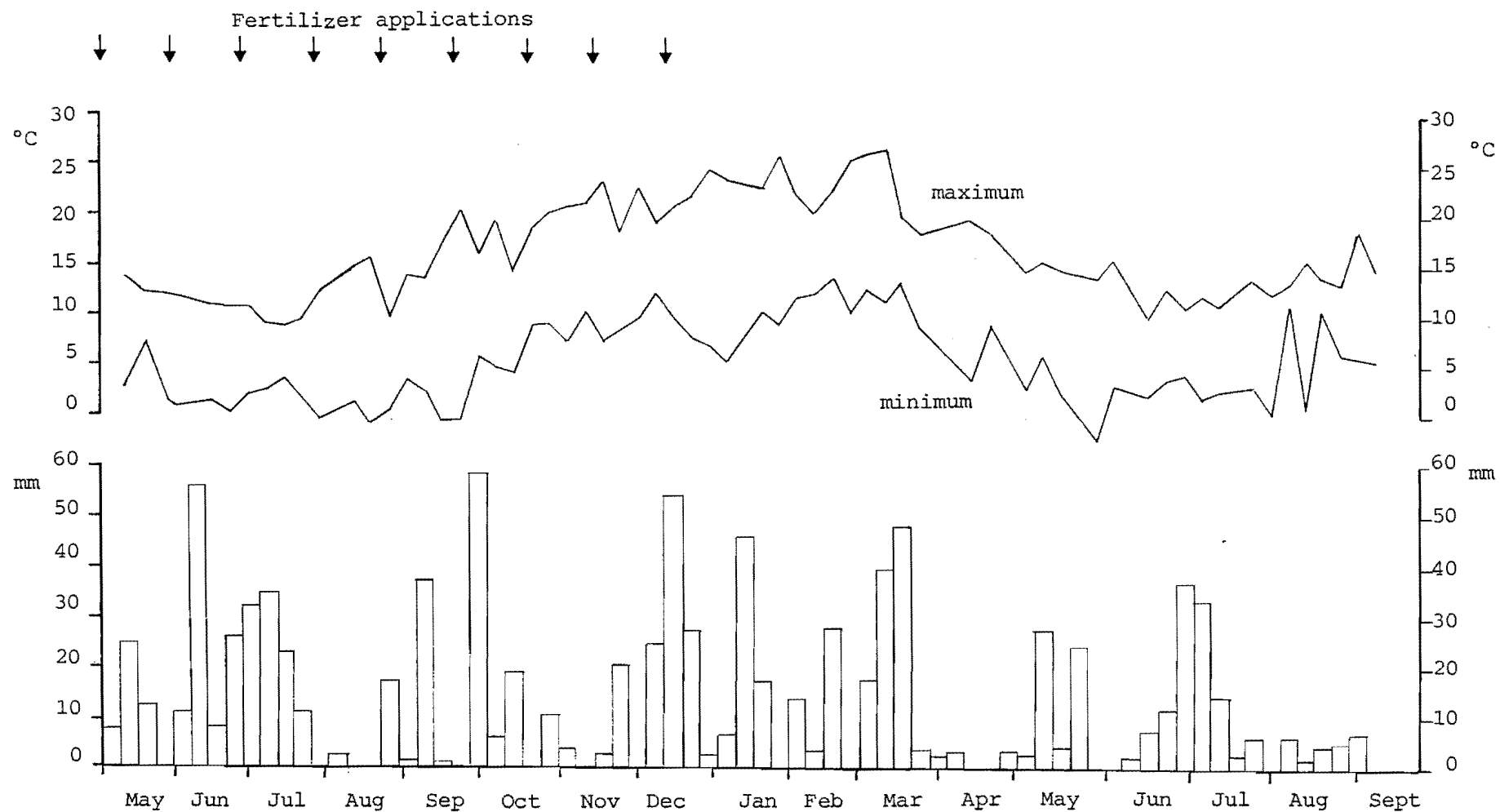


Figure 2.4 Weekly rainfall and average maximum and minimum temperatures

The weather data for Christchurch airport, 12 km inland was also obtained. The long term average rainfall (1950-1980) is 648 mm p.a. The study period was wetter than this with 727 mm at the airport and 775 mm at the study site from May 1983 - April 1984. The summer (Dec-Feb) of 1983/84 was cooler than average, and the winter (Jun-Aug) of 1984 warmer than average.

## 2.8 Outline of Measurements

Measurements were taken on trees and plots to give as comprehensive a picture of growth patterns and fertilizer distribution as possible. Tree growth was monitored by monthly assessments of stem height and diameter, by an intensive foliage sampling scheme and a complete tree harvest at the end of the experiment. These data are presented in Chapter 3.

Chapter 4 looks at patterns of total foliar nitrogen accumulation and distribution in relation to treatment, time and crown position. The status of other nutrients is also briefly discussed.

Chapter 5 describes the movement of fertilizer nitrogen in the system. This was determined primarily by foliar analysis but some supplementary data on volatilization of ammonia, leaching and soil retention are also included.

Following the final harvest a budget of fertilizer nitrogen within the system was drawn up. Recovery of fertilizer within the tree is discussed in Chapter 6. In Chapter 7 the budget is completed by investigating residual fertilizer within the soil. Chapter 8 provides a synthesis of the foregoing material.

## Notes

- (1) Treatment names used in the text are:  
     Main Treatments: Control, Single, 3-Split, 9-Split.  
     Seasonal Treatments: Autumn, Spring, Summer.
- (2) The growing season spans two calendar years resulting in the following names for ages of foliage:  
     1982/83 foliage; formed prior to the experiment starting, also called older foliage (OLFO).  
     1983/84 foliage; formed during the course of the experiment, called 1-year foliage at the final biomass.
- (3) The trees were numbered from 846-867 in accordance with available aluminium tags. Individual tree data are usually restricted to the appendices.

## CHAPTER 3

GROWTH AND BIOMASS RESPONSE TO NITROGEN FERTILIZER IN A 2-YEAR-OLD  
RADIATA PINE STAND

## 3.1 INTRODUCTION

Nitrogen is considered to be a growth limiting factor in many forest ecosystems and consequently the use of nitrogen fertilizers to improve stand productivity has increased in recent decades. It is necessary to know the size of any response in order to assess the economic profitability of fertilization. An understanding of how trees respond to nitrogen is also necessary to predict changes in tree growth patterns and to refine methods of fertilizing forests.

Growth parameters commonly used to assess tree response to fertilizer are height, diameter, basal area, volume and, less regularly, biomass (Bevege 1984). Whilst useful information is obtained, this approach has been criticised by Nambiar (1984b) who considers further advances require an understanding of mechanisms and processes underlying the physiological basis of response to nutrients.

Tree height is unlikely to respond to nitrogen except in very deficient stands (Hunter 1982). In very young stands height is often the only variable assessed but Whyte *et al.* (1978) caution against this and advise a measure of stem diameter also. An index of volume response may be obtained from basal area x height. However, incorporation of a form factor is also recommended (Hunter 1982), as this may change following fertilization (Barker 1978). Stem growth responses are reported for several radiata pine stands in New Zealand (Mead and Gadgil 1978, Woollons and Will 1975, Hunter *et al.* 1985), although where stand density and foliar nitrogen concentrations are high a response is unlikely (Hunter 1982). It is therefore usual to find greatest responses in thinned stands (Mead *et al.* 1984).

Where a biological appraisal of fertilization is important biomass measurements are usually required. Total biomass is useful, but the partitioning of response into various tree components is more valuable in understanding processes. Studies of relative growth often use the allometric relationship for example with root:shoot ratios (Ledig *et al.* 1970). Relative allocation within the crown has also been studied (Will and Hodgkiss 1977, Mead *et al.* 1984). It is generally assumed that nitrogen additions will increase shoot growth relative to root growth (Ledig 1983). A shift in allocation from stems to crown is usually noted, sometimes resulting in loss of apical dominance and bushy, heavy crowns (Will and Hodgkiss 1977, Mead *et al.* 1984).

Net growth responses must occur through the intermediary of



increased photosynthate production. This can be achieved by either increasing the rate of photosynthesis *per se* or the total area of the photosynthetic surface. While both can be important (Miller and Miller 1976, Brix 1972), it is generally considered that the more usual mechanism is an increase in the total leaf area (Linder and Rook 1984, Fagerström and Lohm 1977). This can be achieved by increasing the foliage mass and/or needle longevity.

In a very nitrogen deficient stand of corsican pine (*Pinus nigra* var. *maritima* (Ait) Melv.) needle retention was increased following fertilization (Miller and Miller 1976). Brix (1981) found decreased retention in douglas fir as did Will and Hodgkiss (1977) in radiata pine. Mead *et al.* (1984) found no change in the latter species. Longevity changes may relate to initial nitrogen status of the stand with an increase more likely on very deficient sites (Turner and Olsen 1976).

In conifers foliage mass may be increased by larger individual needles, greater numbers of needles and in species such as pines greater numbers of needles per fascicle. The number and weight of needles were increased following fertilization in douglas fir (Brix 1981). Aronsson *et al.* (1977) showed that the number of needles per fascicle was increased in scots pine following fertilization. However, Nambiar and Fife (in press) report that nitrogen has increased the number and size of needles in radiata pine, but has had no effect on the number of needles per fascicle.

### 3.2 METHODS

Several growth parameters were measured to obtain a comprehensive picture of seasonal patterns and differences between treatments.

#### 3.2.1 Stem

Monthly measurements of height, to the nearest centimetre, and diameters at the base and at 50 cm on the stem were taken to the nearest 0.1 mm. All diameter measurements were made with calipers; two readings were taken at 90° to each other.

#### 3.2.2 Foliage

An intensive foliage sampling scheme was adopted to monitor needle length, needle weight and the number of needles per fascicle. From the beginning of the experiment (May 1983) samples of current foliage were taken fortnightly. For the first three dates the samples consisted of 20 fascicles from the whole crown. Thereafter sampling was restricted to the leader and upper two whorls (Figure 3.1a). Sample size was dictated by the need for sufficient material for later chemical analysis without overly depleting the foliage mass. Two fascicles from the leader and one from each branch were collected, i.e.

fourteen fascicles for most trees.

From November 1983 when the newly initiated foliage became large enough to sample the above scheme was discontinued. The new foliage was sampled at two crown positions (Figure 3.1b):

- (i) Upper crown: Two fascicles from the leader and one from each branch in the upper two whorls arising directly from the stem.
- (ii) Middle crown: One fascicle from each branch arising from the previous year's upper two whorls.

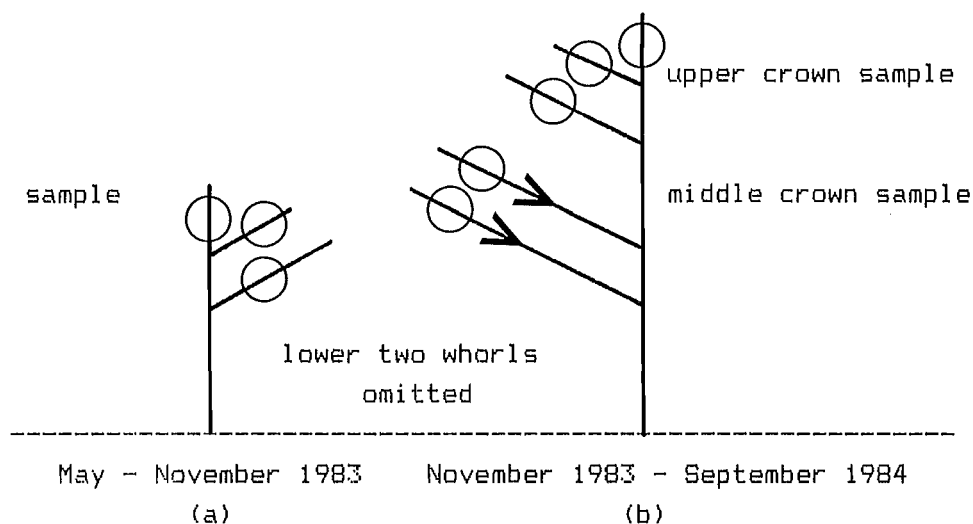


Figure 3.1 Foliage sampling scheme

This sampling continued fortnightly until April 1984 when approximately monthly samples were collected until the end of the experiment. This sampling scheme is very similar to that used by Fife and Nambiar (1982) and in general upper and middle crown designations here can be equated with their primary and secondary order branch needles ( $1^{\circ}\text{BN}$ ,  $2^{\circ}\text{BN}$ ). They noted that the depletion of foliage was unlikely to have influenced growth.

Samples were immediately taken to the laboratory and measured for length to the nearest millimetre. If all the needles in a fascicle were not the same length the longest was recorded. Samples were then dried at  $65^{\circ}\text{C}$  before weighing to the nearest milligram. Mean fascicle weight was determined from the number of fascicles in the sample, and mean needle weight by a division with the mean number of needles per fascicle for each sample.

### 3.2.3 Whole Tree Harvest

3.2.3.1 Above Ground On 9 October 1984 all 22 trees were cut at ground level and transported complete back to the laboratory. Nine above ground components were recognized:

Foliage - current

- 1-year (1983/84 foliage)
- older (1982/83 foliage)

Twigs - current

- 1-year
- older

Stem - 1-year

- older - wood
- bark

Current material was from the new flush and was <1 month old. 1-year material was formed during the 1983/84 season. Older foliage is predominately of 1982/83 origin with a small proportion of 1981/82 foliage. Older wood and twigs were not sectioned into annual increments and so contain wood formed in each year of growth including a sheath of 1-year growth.

The branches were cut from the stem as above and dried initially with the foliage intact. This meant subsequent stripping of foliage was easy to give individual components which were dried at 65°C prior to weighing. The initial drying could have caused some retranslocation of nutrients between twigs and foliage.

The 1-year stem (1983/84 leader) was removed and dried. The remainder of the stem was weighed fresh, then 4 cm thick discs were cut at 30 cm intervals. The bark (including phloem and cambium) was peeled from each disc. Wood and bark were weighed separately then dried at 65°C. Whole stem weights were calculated using ratios of green to dry weight. All components were weighed to the nearest gram after drying.

**3.2.3.2 Below Ground** From 23 October to 19 November the root systems were extracted. First 2-3 m deep trenches were dug around half of each plot with a mechanical digger. The plastic sheeting was then removed to expose a dense mat of roots (Figure 3.2) which were easily cut off and placed in bags. Initially a fire hose was used to wash away the sand and expose the root system. This method proved to be unsatisfactory and was discontinued after four complete plots (one of each main treatment) had been tackled. The sand has a bulk density of 1.5 g/cm<sup>3</sup> and proved resistant to the water jet. However, the main objection to this method was the loss of fine roots which has required a correction to be made for the four plots extracted in this manner (Appendix 1).

The final method adopted was to pass the complete volume of sand through a wire screen (mesh size 2 cm<sup>2</sup>) and pick off the roots (Figure 3.3). Because of the time involved only half of each plot was excavated down to the depth of the plastic, i.e. 75 cm. The majority of roots

were found within the top 50 cm of soil, with the exception of the mat around the plastic (Figure 3.2).



Figure 3.2 Root mat of fertilized tree, exposed after removing plastic.



Figure 3.3 Root excavation.

The whole rootstock and tap root were removed and any sinker roots penetrating below 75 cm were excavated as best as possible. Sinker roots were unusual and so losses would have been minimal. All roots were returned to the laboratory and placed in cold storage prior to sorting.

Unlike some proposals for mechanical excavation of roots (e.g. Evelyn 1662 p.32), this method was time consuming and laborious (Bullsmith, Clarke, Fuller, Schasching and Young pers. comm.). It was however considered justified because of the apparent high recovery of fine roots.

A standard terminology for tree roots does not exist (Santantonio and Hermann 1985). Some workers have adopted the classification advocated by Bohm (1979) where fine roots are those <2 mm diameter (e.g. Jackson and Chittenden 1981, Persson 1978, Grier *et al.* 1981). However, this subdivision is arbitrary in that it does not account for morphology and function. Its adoption is criticised by Santantonio (pers. comm.) who uses <1 mm as do Ford and Deans (1977) and Kohmann (1972). Divisions as low as 0.5 mm have been used (Squire *et al.* 1978). In this study fine roots are defined as <2 mm in order to be compatible with previous studies on radiata pine in New Zealand (Jackson and Chittenden 1981, Clinton 1986).

Three components of the below ground system were recognised:

Rootstock (below ground extension of the stem)  
Coarse roots (including tap root) >2 mm  
Fine roots <2 mm

The initial laboratory sorting procedures are outlined in Figure 3.4. All laterals and the tap root were cut from the rootstock. Coarse roots were separated from fine roots using a 2 mm slot gauge. The rootstock and coarse roots were dried at 65°C, brushed free of sand and weighed to the nearest gram.

A large mass of "wet fine roots" remained. The bulk of sand was removed using a 0.5 mm sieve. This sand included the finest root and organic matter fragments and was retained to check its nitrogen status. The remaining mass of "fine roots" was oven dried and weighed. At this stage there was still significant contamination by sand and organic matter. This had to be corrected for to give an accurate fine root biomass and removed to enable nitrogen analysis to proceed. These corrections were done on four subsamples, two for nitrogen analysis, and two for fine root biomass estimation (Figure 3.5).

The use of a 2 mm sieve, to initially sort these subsamples, was purely for convenience to give a fraction from which most of the organic matter could be separated. In fact roots were removed rather than organic matter, which is considered to be a more positive approach and eliminates some contamination (Jackson and Chittenden 1981). The material passing through the 2 mm sieve then went on to one of 0.5 mm. The retained material contained fine root and organic matter fragments.

The retained material contained fine root and organic matter fragments. Where the latter constituted a minor component it was picked out and discarded. In other cases a further subsample was required to correct for this organic matter contamination.

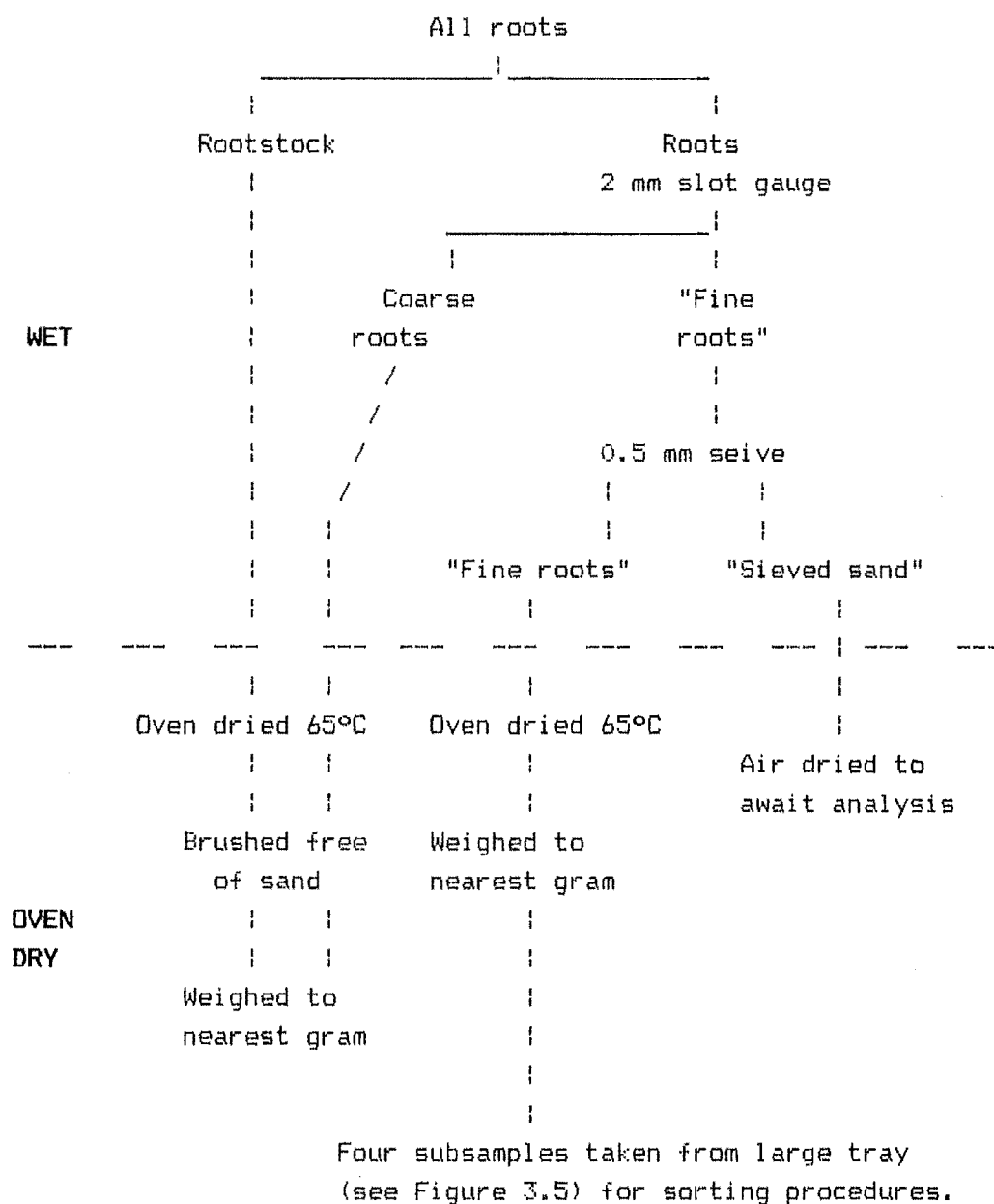


Figure 3.4 Initial laboratory sorting procedures for roots

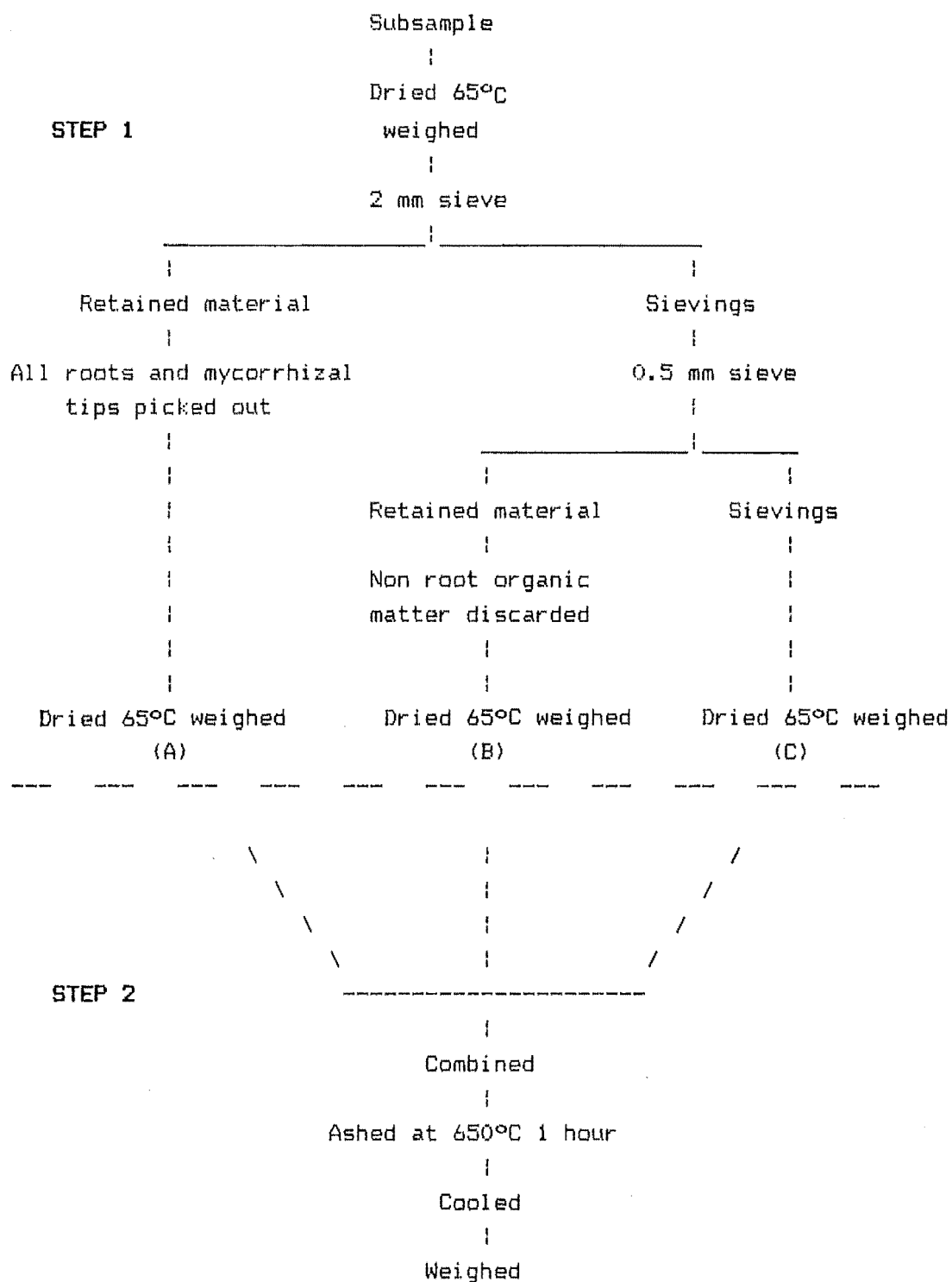


Figure 3.5 Method adopted for sorting two subsamples to estimate fine root biomass.

At the end of Step 1, the original subsample minus the organic matter contamination was left. The percentage of organic matter (% O.M.) contamination in the subsample was calculated:

$$\% \text{ O.M.} = \frac{\text{Initial weight subsample} - (A + B + C) \times 100}{\text{Initial weight subsample}}$$

The sand contamination was then calculated by ashing the subsample:

$$\% \text{ sand contamination} = \frac{\text{weight after ashing} \times 100}{(A + B + C)}$$

The sorting procedure for the two subsamples for nitrogen analysis is the same for Step 1. Then A and B are combined to give the fine root sample for analysis. C was retained separately to check its nitrogen status.

The correction for fine root biomass from the original oven dried "fine root" mass (Figure 3.4) is illustrated in the following example:

Tree 855: weight of oven dry "fine roots" including sand and organic matter contamination = 2451 grams

Subsample	Weight g	% O.M.	% sand
1	12.38	9.3	69.5
2	8.06	8.2	66.5
3	11.82	10.0	
4	15.62	9.7	
		-----	-----
		$\bar{x}$ 9.3	$\bar{x}$ 68.0

$$\text{Correction: } 2451 \text{ g} - 9.3\% = 2223 \text{ g} - 68\% = 711 \text{ g}$$

The weight of coarse roots (excluding tap root) and fine roots were doubled to give a figure for the whole plot. The estimate obtained in this way assumes a symmetrical distribution of roots. Observation of laterals emanating from the rootstock generally supported this which agrees with Nambiar (1983).

### 3.3 RESULTS AND DISCUSSION

#### 3.3.1 Height

Mean tree heights at the beginning of the experiment (May 1983), after one growing season (June 1984) and at the end of the experiment are presented in Tables 3.1 and 3.2. An analysis of co-variance for the main treatments, using initial height as the co-variate, showed no significant height response to 90 g N (Table 3.1). Similarly an analysis of variance for the seasonal treatments showed no response to



30 g N (Table 3.2). The lack of a response is in agreement with Hunter (1982).

The seasonal pattern of height growth at Bottle Lake is illustrated in Figure 3.6a along with data from two other studies collated by Jackson *et al.* (1976). Monthly increment was calculated as 30 day intervals from June 1983 to June 1984 when the average height for all trees increased from 1.62 to 2.84 m. Height growth is not continuous at Christchurch and starts later than the other sites. It culminates later and is generally more evenly spread over the year. In the second growing season after treatment height growth commenced in August.

Table 3.1 Mean tree heights for the main treatments at the start of the experiment, the end of one growing season and at harvesting.

	Control	Single	3-Split	9-Split	(SE)	P
	(m)					
Initial Height (May 1983)	1.62	1.66	1.66	1.52	(0.05)	0.242
June 1984*	2.78	2.95	3.12	2.91	(0.09)	0.155
October 1984*	3.14	3.27	3.42	3.20	(0.11)	0.410

(SE): standard error (see Appendix 15).

P : probability of treatment differences according to ANCOVA.

\* : adjusted means from co-variance analysis, with initial heights (see Appendix 15).

Table 3.2 Mean tree heights at the beginning, after one growing season and at the end of the experiment for the seasonal treatments.

	Control	Autumn	Spring	Summer	(SE)	P
	(m)					
Initial Height (May 1983)	1.62	1.45	1.66	1.54	(0.054)	0.108
June 1984	2.78	2.54	2.82	2.51	(0.179)	0.495
October 1984	3.15	2.90	3.28	2.73	(0.210)	0.311

(SE): standard error.

P : probability of treatment differences according to ANOVA.

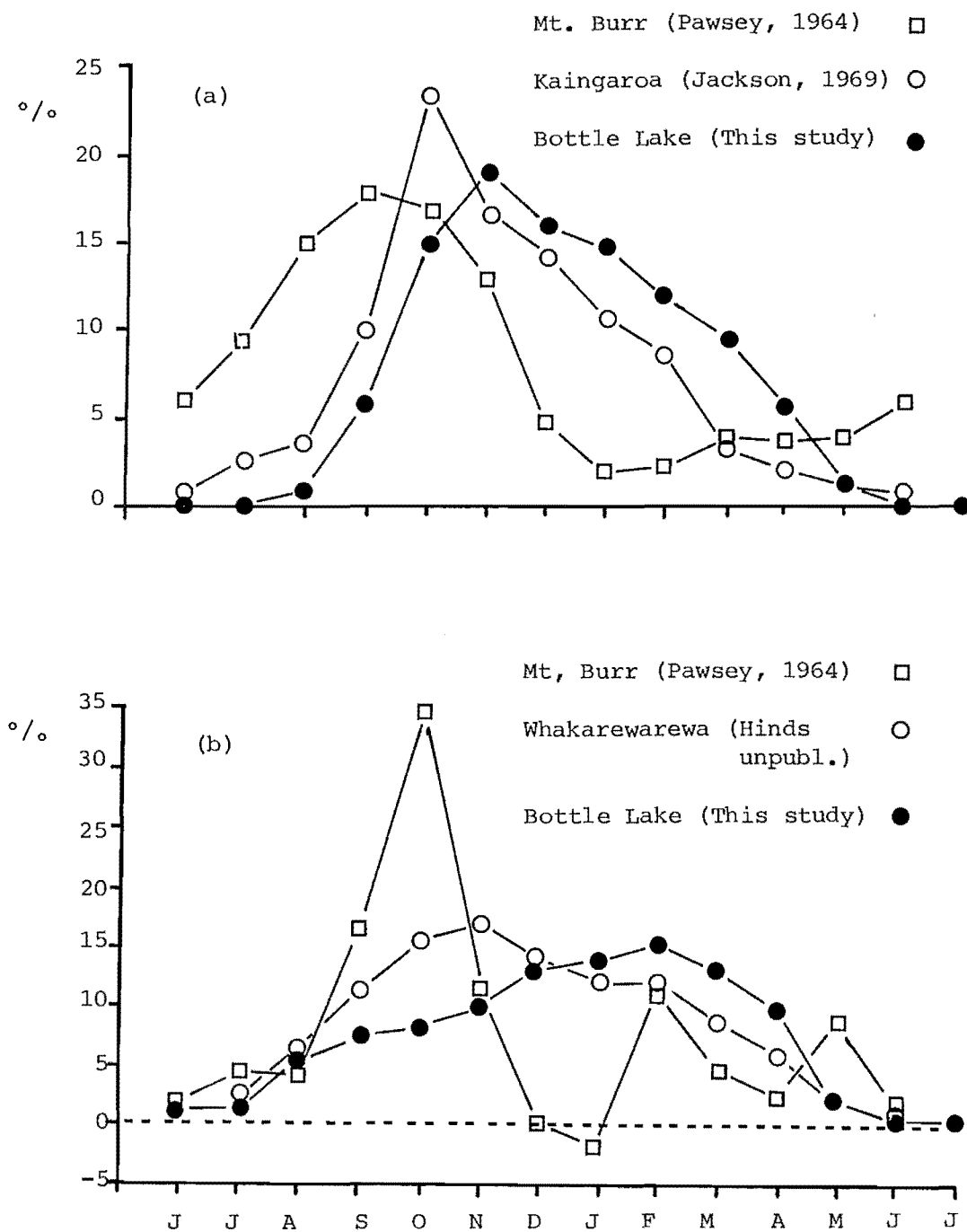


Figure 3.6 Monthly height (a) and diameter increment (b) for radiata pine calculated as percent of annual total (source: Jackson et al., 1976)

### 3.3.2 Stem Diameter

The seasonal pattern of diameter increment at the base of the stem in comparison with two other sites is shown in Figure 3.6b. Diameter growth peaks later than height growth and later than on the other sites. Growth ceases during the winter at Christchurch.

Stem diameters at the base and at 50 cm were analysed for treatment differences using analysis of co-variance for the main treatments. Initial diameter and its square were tried as co-variates. Both gave very similar results and the former is presented here (Tables 3.3 and 3.4). For the seasonal treatments analyses of variance were run for data at the end of the growing season and the end of the experiment (Table 3.5).

Table 3.3 Stem diameter over bark at the base for the main treatments, adjusted for initial diameter.

	Control	Single	3-Split	9-Split	(SE)	P
	(mm)					
May 1983 (initial)	40.3*	39.9	41.2	38.8	(0.20)	0.861
December	54.3	55.2	56.6	55.8	(0.91)	0.401
January 1984	59.1	61.2	62.6	62.5	(1.04)	0.120
February	63.1	65.4	67.0	66.0	(1.38)	0.280
March	67.3	72.1	73.6	73.0	(1.21)	0.015
April	71.2	77.2	78.9	78.7	(1.43)	0.009
May	75.3	79.4	81.0	82.4	(1.63)	0.052
June	74.3	79.3	81.9	81.3	(1.63)	0.028
August	76.0	80.0	82.7	82.5	(1.68)	0.054
September	81.0	84.3	87.3	88.4	(1.72)	0.047

\* : unadjusted means in May 1983; adjusted means for later dates.  
(SE): standard error.

P : probability of treatment differences according to ANCOVA.

Table 3.4 Stem diameter over bark at 50 cm for the main treatments, adjusted for initial diameter.

	Control	Single	3-Split	9-Split	(SE)	P
	(mm)					
May 1983 (initial)	28.2*	28.0	30.5	28.2	(0.15)	0.590
December	41.8	41.5	42.1	40.9	(1.18)	0.906
January 1984	46.3	47.1	47.6	47.2	(1.20)	0.890
February	48.8	50.2	51.2	50.6	(1.24)	0.604
March	53.8	55.9	57.6	57.2	(1.28)	0.225
April	57.3	59.6	61.5	61.0	(1.31)	0.184
May	59.6	61.6	63.7	63.7	(1.42)	0.199
June	59.6	61.8	64.4	63.7	(1.33)	0.115
August	60.7	62.5	65.1	64.5	(1.54)	0.250
September	65.6	67.4	70.2	70.4	(1.60)	0.176

\* : unadjusted means in May, 1983; adjusted means for later dates.

(SE): standard error.

P : probability of treatment differences according to ANCOVA.

Table 3.5 Stem diameter over bark at the base for the seasonal treatments.

	Control	Autumn	Spring	Summer	(SE)	P
	(mm)					
Initial May 1983	40.3	36.3	40.1	38.8	(2.47)	0.620
June 1984	74.6	69.8	79.5	76.0	(2.10)	0.083
September 1984	81.3	74.9	85.4	81.3	(2.27)	0.082

(SE): standard error.

P : probability of treatment differences according to ANOVA.

Statistically significant increases in basal diameter were apparent by March (Table 3.3). An analysis using single degree of freedom contrasts shows the difference to be due to fertilizer with no effect of split applications (Appendix 2). The treatment differences at 50 cm up the stem were much less pronounced (Table 3.4). A similar analysis of contrasts shows significant effects due to fertilizer at  $P < 0.1$  (Appendix 2). The differences between seasonal treatments (Table 3.5) were attributed primarily to initial diameter differences. A co-variance analysis between the pooled seasonal data ( $n = 6$ ) and the Control in June showed there had been no statistically significant response to 30 g N (adjusted means; Control=78.7 mm, Seasonal=76.0 mm).

In conclusion then, there was a highly significant diameter response to 90 g N, but none to 30 g N. This response was primarily at the base of the stem, being rather weak at 50 cm. The magnitude of the response at the end of the first growing season (June, 1984) was 8.8% at the stem base. Nambiar and Cellier (1985) have recently reviewed the response of young radiata pine to nitrogen fertilizers in South Australia. They reported small (<10%) responses and cast doubt on the efficiency of the intensive nitrogen fertilization programme in the area. The continuation of these small responses through to maturity was considered unlikely. However, stem diameter in their trials was measured at 15 cm above ground level. The pattern of diameter response at Bottle Lake suggests that the below ground portion of the stem may be responding with possible benefits in terms of anchoring the young tree and promoting a larger root system. In a more recent study from South Australia, Nambiar and Bowen (1986) report a 12% diameter increase in 4.8-year-old trees following nitrogen fertilization at age 1. The response increased over time, which suggests that further increases might have been expected at Bottle Lake in subsequent years.

### 3.3.3 Biomass

The biomass of the trees (Tables 3.6 and 3.7) was compared with previous studies, analysed for treatment differences and for allocation patterns in response to fertilizer.

**3.3.3.1 General Observations** The total biomass at the end of the experiment (age 3.1 years) varied from 6.1–13.4 kg per tree (Appendix 3). Trees fertilized with 90 g N had an average biomass of 11 kg; an increase of 30% over controls at 8.5 kg (Table 3.6). Trees fertilized with 30 g N had similar dry weights to the Control, and averaged 8.2 kg per tree (Table 3.7). The above ground biomass of trees at the beginning of the trial was estimated by felling six trees in August, 1983, in a similar size range (Appendix 4). The increase during the study from 0.9 to 5.9 kg per tree clearly shows an exponential phase of growth (Figures 3.7 and 2.1).

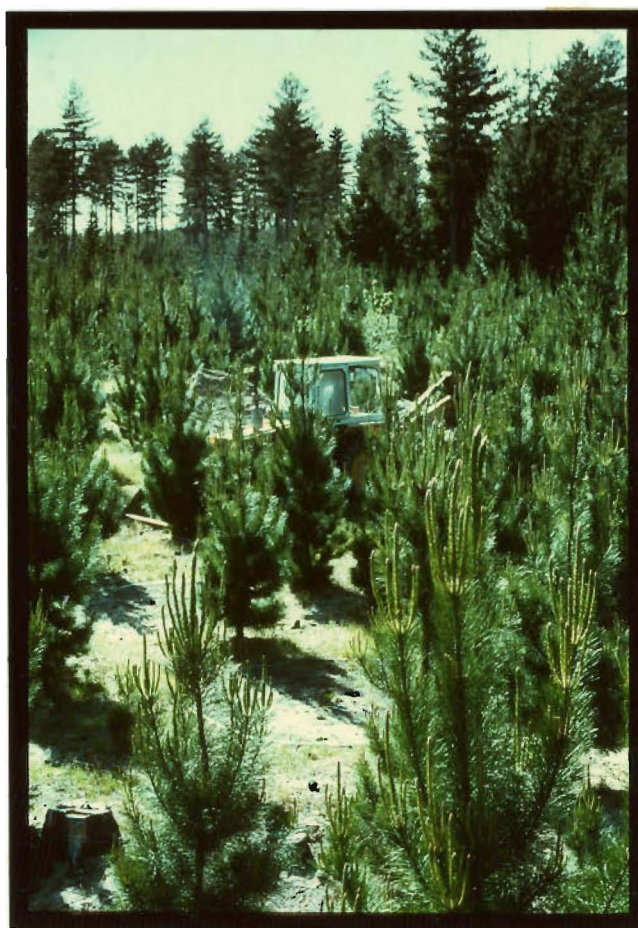


Figure 3.7 The site in October 1984 at the end of the experiment.

Table 3.8 gives comparative data for above ground biomass drawn from studies at various sites in New Zealand and Australia. Biomass has been expressed as kilogrammes per tree, necessitating a conversion from tonnes per hectare given in most studies. There is considerable variation, reflecting site and possibly planting stock quality. Bottle Lake is at least as productive as the Kaingaroa site during the establishment phase (Madgwick *et al.* 1977). Productivity compares favourably with Spehrs plantation in South Australia (Fife and Nambiar *in press*), but is not as productive as at Mount Gambiar with added fertilizer (Fife and Nambiar 1982). Bottle Lake is considerably more productive than the other sites listed.

There is a dearth of information for the below ground biomass of radiata pine (Madgwick 1985), particularly for fine roots. Comparative studies are further hindered by the variable size definition of fine roots (Nambiar 1981). The biomass of fine roots ( $<2$  mm) in this study is 739 g/tree on Control plots. Assuming conversion to an areal basis is valid, this is equivalent to 1660 kg/ha. Moir and Bachelard (1969) estimate standing crop of fine roots ( $<3$  mm) to be 3450, 3010 and 2110 kg/ha for age 10, 20 and 30-year-old radiata pine. Clinton (1986)

gives 655 kg/ha for roots <2 mm in a 14-year-old radiata pine stand; however, this refers only to the top 10 cm of soil. Values of 1-3 kg fine roots per tree are common in the literature (Safford and Bell 1972, Santantonio *et al.* 1977); although these are usually for older trees.

Table 3.6 Dry weight of tree components at the end of the experiment (Oct. 1984) for the main treatments.

	Control	Single	3-Split	9-Split	(SE)	P
	grams					
-----						
Foliage						
current	156	151	154	137	(24)	0.947
1-year	1571	2002	2196	1696	(146)	0.040
older	561	602	688	435	(60)	0.069
Twigs						
current	193	216	235	203	(24)	0.664
1-year	707	921	914	883	(87)	0.311
older	718	911	984	803	(131)	0.523
Stems						
1-year	241	230	334	278	(47)	0.439
older wood	1439	1627	1773	1593	(112)	0.271
older bark	295	343	342	327	(28)	0.609
-----						
Above Ground	5881	7003	7620	6355	(470)	0.100
-----						
Roots						
stock	811	1098	934	1144	(86)	0.064
coarse	1002	1604	1584	1564	(173)	0.084
fine*	739	1210	1599	1157	(119)	0.002
-----						
Below Ground	2552	3912	4117	3865	(241)	0.002
-----						
TOTAL	8433	10915	11737	10220	(611)	0.015
-----						
Initial						
D <sup>2</sup> H	26.48	26.46	29.08	23.58	-	-
Root:shoot						
ratio	0.434	0.559	0.540	0.608	-	-
-----						

\* : one replicate from each treatment is an estimate as calculated in Appendix 1.

(SE): standard error

P : probability of differences according to ANOVA.

Table 3.7 Dry weight of tree components at the end of the experiment (Oct. 1984) for the seasonal treatments.

	Control*	Autumn	Spring	Summer	(SE)	P
	grams					
Foliage						
current	156	142	180	89	(29)	0.224
1-year	1571	1267	1859	1460	(277)	0.416
older	561	355	542	496	(112)	0.543
Twigs						
current	193	213	230	122	(10)	0.009
1-year	707	649	666	609	(98)	0.916
older	718	638	754	807	(218)	0.861
Stem						
1-year	241	160	283	106	(48)	0.162
older wood	1439	1135	1439	1163	(138)	0.356
older bark	295	287	267	211	(32)	0.346
Above ground	5881	4846	6220	5063	(742)	0.468
Roots						
stock	811	669	877	741	(143)	0.627
coarse	1002	981	1365	1209	(249)	0.604
fine	739	537	1073	953	(203)	0.290
Below ground	2552	2187	3315	2903	(492)	0.383
TOTAL	8433	7033	9535	7966	(1211)	0.441
Initial						
D <sup>2</sup> H	26.48	19.22	26.63	23.75	-	-
Root:shoot						
ratio	0.434	0.451	0.533	0.573	-	-

\* : Control with four replicates is reproduced for comparison  
(SE): standard error.

P : probability of treatment differences according to ANOVA.



Table 3.8 Comparative data for above ground biomass of radiata pine in Australia and New Zealand.

Site	Age (yrs)	gN/ tree	Ht (m)	Foliage ----- kg/tree	Branches ----- kg/tree	Stem ----- kg/tree	Total ----- kg/tree	Reference
Bottle lake	2	0	1.60	0.48	0.16	0.26	0.90	This Study
N.Z.	3	0	3.15	2.29	1.62	1.97	5.88	
	3	90	3.29	2.69	2.02	2.28	6.99	
Kaingaroa	2	0	1.05	0.14	0.05	0.09	0.28	Madgwick <i>et al.</i>
N.Z.	4	0	3.91	3.05	2.79	3.60	9.44	1977
Mt. Gambiar								Fife & Nambiar
SA	3	35	3.34	3.96	2.57	2.93	9.46	1982
Spehrs Pltn.	4	0	3.89	2.26	1.60	2.26	6.12	Nambiar & Fife
SA	4	80	4.47	4.97	3.31	4.47	12.89	(in press)
Tumut For.	3	0	1.4	0.34	0.13	0.27	0.74	Forrest and
NSW	5	0	3.1	1.41	0.80	1.61	3.82	Ovington 1970
Belanglo	4	0	1.21	0.11	0.03	0.08	0.22	Snowdon and
For. NSW	4	26+P	2.22	0.38	0.15	0.45	0.98	Waring 1985
Eyrewell	4	0	2.22	1.07	0.54	0.74	2.35	Grottker 1984
N.Z.								

The fine root biomass determined in this study may be compared with a published equation for predicting radiata pine root mass (Jackson and Chittenden 1981). Their equation (4) is:

$$(\text{total fine root o.d.wt.}) = -121.35 + 0.526 (\text{foliage o.d.wt.})$$

If applied to the control tree data (Table 3.6), the estimated weight of fine roots would be 1082 g which is a 46% overestimate. If applied to the pooled data for trees fertilized at 90 g N, the estimated value is 1309 g which agrees very well with the actual value of 1322 g. Clearly this equation is inappropriate for Control trees in this study. This highlights the possible problems in using a single estimating equation across nutritional treatments (c.f. Snowdon 1985). The above equation was developed on trees 3-8 years old, grown in a long trench. The soil was a pumiceous sandy loam but no details were given of its fertility or of the tree's nutritional status.

3.3.3.2 Treatment Differences Analyses of variance for the main treatments show statistically significant ( $p < 0.05$ ) differences for 1-year foliage, total and below ground biomass, particularly the fine roots (Table 3.6). The analyses for seasonal treatments generally show no significant differences (Table 3.7). The analyses were hindered by initial tree size differences. Accordingly a co-variance analysis was run for the main treatments to remove this effect using initial D<sup>2</sup>H as a co-variate (Table 3.9). The use of this and other possible co-variates is discussed in Appendix 5. All the components listed in Table 3.9 show highly significant responses to nitrogen according to single degree of freedom contrasts (Appendix 6). There were no statistically significant differences between Single and Split applications.

Table 3.9 Dry weight of selected tree components at the end of the experiment (Oct. 1984), adjusted for initial tree size differences; main treatments.

Biomass component	Control	Single	3-Split	9-Split	(SE)	P
	----- Dry weight grams -----					
TOTAL	8422	10906	11371	10606	(444)	0.003
Above ground	5872	6994	7278	6715	(236)	0.009
Below ground	2550	3912	4094	3890	(250)	0.004
1-yr foliage	1568	2000	2104	1793	(100)	0.015
Stem*	1973	2198	2354	2298	(107)	0.119
Older stem wood	1437	1626	1702	1668	(77)	0.129
1-yr wood#	947	1150	1205	1207	(64)	0.044
Rootstock	809	1098	905	1174	(83)	0.040
Coarse roots	1002	1605	1617	1529	(177)	0.094
Fine roots+	738	1209	1571	1187	(120)	0.004

\*: Stem = summation of older wood and bark and 1-year stem, see Table 3.6.

#: 1 year wood = 1 year twigs and 1 year stem, excluding the non-sampled sheath of 1 year wood on older twigs and stem.

+: The analysis refers to a full four replications per treatment. However, this includes one estimated value from each treatment (Appendix 1). These should not be treated as "normal" samples and so the degrees of freedom should be reduced. Subtracting 4 df has the effect of lowering the F value from 7.89 to 5.02 with the significance level becoming  $0.025 < p < 0.05$ .

The 30% response in total biomass is not allocated evenly across tree components. The response is 19% above ground and 55% below ground. There is a similar response of 25% in the 1-year foliage and 1-year wood. All below ground components have responded and in particular fine

roots, with an 80% increase over controls (Table 3.9).

The seasonal treatment data (Table 3.7) was not amenable to co-variance analysis because of the low replication. However, in view of the non-significant ANOVA differences between seasonal treatments or between Single and Split applications, all the data were pooled in an analysis of co-variance across rates (0, 30 and 90 grams) of applied nitrogen (Table 3.10). There was a highly significant above ground response to 90 g N, but none to 30 g N. In the co-variance analysis for below ground biomass the individual treatment regression slopes were significantly different. Accordingly these regressions were plotted and compared with those for above ground (Figure 3.8). Clearly above ground biomass increases in accordance with initial tree size, which is statistically the same across treatments. At the two lower rates of nitrogen (0 and 30 grams) the trend below ground is the same. However, with 90 g N there is virtually the same below ground biomass regardless of the initial tree size. This is discussed in the following section on root:shoot interactions. There is an indication from Figure 3.8 that there was in fact a response below ground to 30 g N. This was confirmed by an analysis of co-variance, the adjusted means being 2353 and 3024 grams for Control and seasonal treatments respectively.

Table 3.10 Dry weight of selected tree components at the end of the experiment (Oct, 1984), adjusted for initial tree size differences: effect of nitrogen rate.

Tree component	Grams of nitrogen applied			(SE)	P
	0	30	90		
	n=4	n=6	n=12		
	----- dry weight grams -----				
Total	8225a*	8575a	10769b	(421)	0.000
Above Ground	5717a	5692a	6848b	(232)	0.000
Below Ground	regression slopes significantly different P=0.016				
1-year Foliage	1518a	1623a	1917b	(105)	0.005
Fine Roots	711a	907a	1297b	(131)	0.002

(SE): standard error for n=4.

P : probability of treatment differences according to ANCOVA.

\* : means followed by the same letter are not significantly different at  $P < 0.05$  according to Duncan's test.

Some care is required in comparing responses to 30 and 90 g N (Table 3.10). The co-variance analysis was not perfect as demonstrated by the different results if older foliage is the co-variate (Appendix 5). With either co-variate the increase in 1-year foliage over Controls was not proportional to the quantity of nitrogen added. This was not a problem for fine roots because co-variance analysis had very

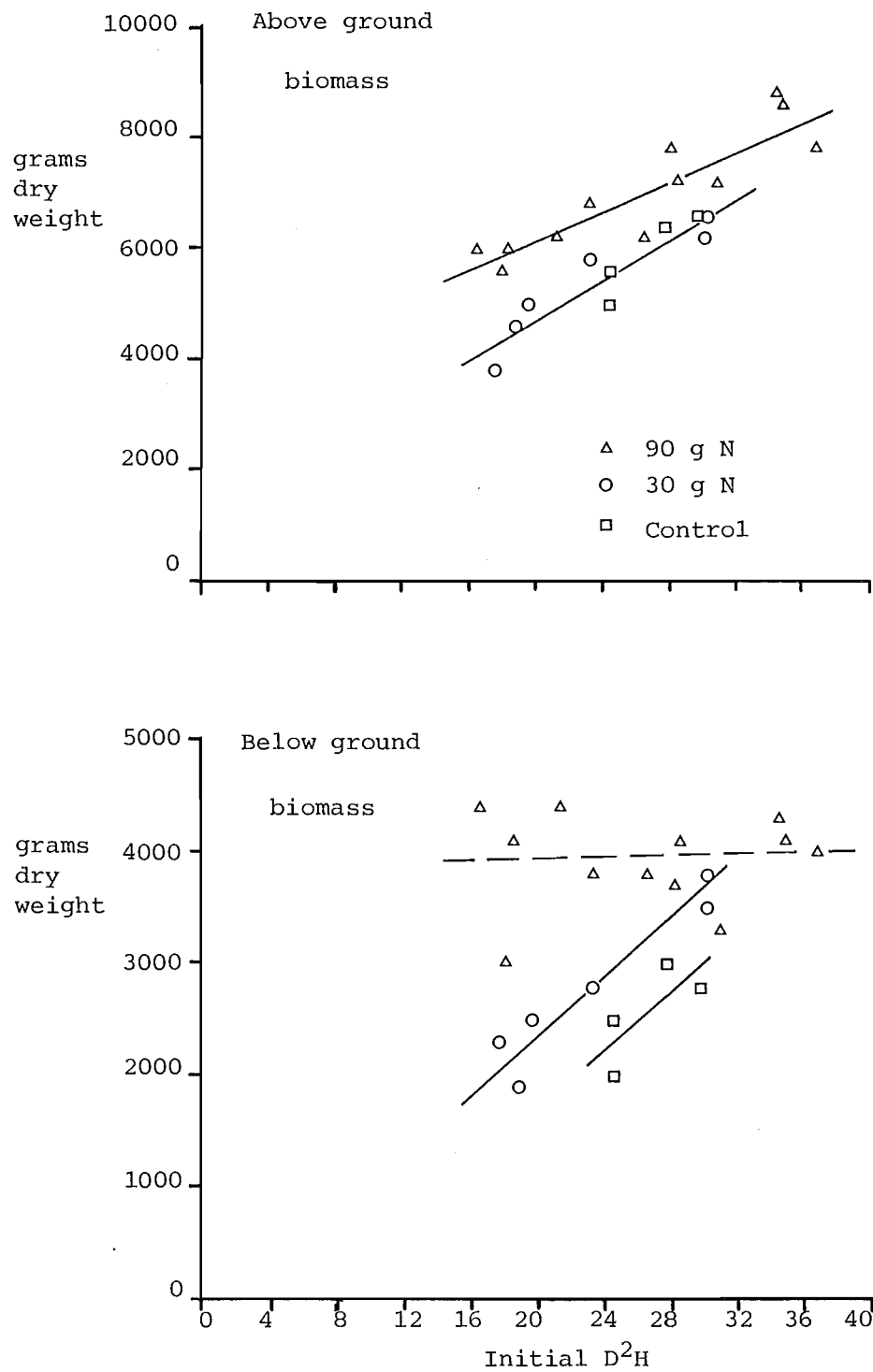


Figure 3.8 Relationship between initial tree size and final biomass

little effect, there being a very poor overall relationship between initial D<sup>2</sup>H and below ground biomass (Figure 3.8). It is apparent that the increase in fine roots over Controls is in proportion to the quantity of nitrogen applied (Table 3.10).

Nitrogen has increased foliage biomass which in turn allows a greater production of photosynthate. This has been channelled into extra production of stems, twigs and roots. The increased photosynthate production could be attributed to the increased foliage mass alone or in conjunction with an increased efficiency of photosynthesis.

Photosynthetic efficiency (see Linder and Rook, (1984) for a review) was not measured in this study. Instead of direct measures, some workers have calculated net assimilation rates as dry weight increases per unit of foliage (Miller and Miller 1976, Brix 1983). Given that foliage itself is a major component of the year's production and given the limited duration of the experiment, this type of analysis was considered inappropriate here.

**3.3.3.3 Root:Shoot Interactions** It is widely believed there is a functional equilibrium between the size and activity of roots and shoots (Brouwer 1983, Cannell 1985, Ledig 1983). There has been considerable research in this area but mainly on agronomic crops. The obvious difficulties in excavating root systems have generally restricted forest researchers to seedlings (e.g. Ledig *et al.* 1970, Carlson and Presig 1980). There have been few studies on tree root systems in relation to shoot growth and as already noted there is a dearth of information for radiata pine.

Reported root:shoot ratios for radiata pine are given in Table 3.11. The value in this study (from Table 3.6) is higher than previous

Table 3.11 Comparative data for root:shoot ratios in radiata pine.

Site	Age (yrs)	Root:shoot ratio	Roots collected	Reference
Chch. N.Z.	3	0.434*	all	This Study
Kaingaroa N.Z.	18	0.12	all#	Will 1966
Rotorua N.Z.	6	0.22*	>5mm	Will & Hodgkiss 1977
Belanglo N.S.W.	4	0.167*	>3mm	Snowdon & Waring 1985
Waitarere N.Z.	4	0.213	all#	Ritchie 1968
	15	0.278		
	28	0.400		
Mt.Stromlo A.C.T.	8	0.19	>5mm	Ovington <i>et al.</i> 1967
Fingal, Tasmania	3+	0.25	all	Nielsen <i>et al.</i> 1984

\*: value for control treatment in fertilizer experiments.

#: fine roots by sampling.

+: seedlings.

estimates. The wide range of values must in part be due to the excavation methods adopted. There is some controversy in the literature as to the direction of root:shoot ratio as trees age or indeed whether it does vary. Ledig *et al.* (1970) report an increasing root:shoot ratio during the seedling development stage in loblolly pine. Conversely Carlson and Presig (1980) show that root:shoot ratio to decrease in douglas fir, and assume that this trend continued with older trees, quoting Eis (1974) and Ovington (1957). This contention is debatable given the difficulty in completely recovering large root systems. Madgwick (1985) utilised estimating regression equations for above and below ground components to show a theoretically increasing root:shoot ratio from DBH =10 cm to DBH = 40 cm. This is supported by Ritchie (1968) (Table 3.11).

The response of roots to nitrogen fertilizer (Table 3.6) is not unusual (Kohmann 1972, Nambiar 1980), but the relatively greater response below ground contradicts the widely held view that nitrogen decreases root:shoot ratio (Ledig 1983, Nambiar 1980). The considerable literature supporting this conventional wisdom has led to models of root:shoot interactions based on nitrogen supply (Thornley 1977, McMurtrie 1985). In accordance with these views it is usually assumed that on infertile sites (particularly those deficient in nitrogen) a greater proportion of net primary production is allocated below ground (Linder and Axelsson 1982, Keyes and Grier 1981, Tamm 1979). This trend was apparent in a trial with radiata pine (Will and Hodgkiss 1977) where root:shoot ratio decreased from 0.22 in a low nitrogen treatment to 0.16 in a high nitrogen treatment. However, the method of root estimation was rudimentary with no roots < 5 mm being collected.

There are, however, opposing views in the literature, e.g. Nadelhoffer *et al.* (1985) suggested that fine root production increased along with nitrogen availability and that the ratio of root to shoot production may have increased. In this study nitrogen has caused a significant ( $p=0.017$ ) increase in root:shoot ratio (Table 3.12). The same analysis using logarithmically transformed data (c.f. Snowdon and Waring 1985) gave a significance level of  $p=0.0099$ .

Table 3.12 Root:shoot ratios as influenced by the rate of nitrogen applied.

Treatment	g N	Root:shoot ratio	Number of trees
Control	0	0.434	4
Seasonal	30	0.519	6
Main	90	0.569	12

However, Ledig *et al.* (1970) caution against the use of simple root:shoot ratios because they change with age (tree size). They suggest the use of the allometric relationship:

$$\ln (\text{Shoot wt.}) = a_0 + a_1 \ln (\text{Root wt.}).$$

Real treatment differences are ascertained by comparing the coefficient,  $a_1$  (slope) between individual treatment regressions. If  $a_1 < 1$ , then a proportionately greater increase in roots than shoots is occurring. Adoption of this analysis often alters conclusions arrived at from examining root:shoot ratios (Waring 1950, Ledig and Perry 1965).

If there is a natural increase in root:shoot ratio with tree size, then the trend in Table 3.12 could be a case of promoting the fertilized trees along the growth curve (c.f. Miller 1981). This was tested on the four Control trees by regressing root:shoot ratio on total biomass. There was a positive relationship ( $r^2 = 0.62$ ) which indicated that for trees of this age, on this site, root:shoot ratio increases with tree size. An allometric analysis of the data was therefore appropriate (Table 3.13).

Table 3.13 Regression equations for the allometric relationship of root on shoot: as influenced by the rate of nitrogen:  
 $\ln \text{Shoot (wt.)} = a_0 + a_1 \ln \text{Root (wt.)}$ .

Treatment	g N	$a_0$	(SE)	$a_1$	(SE)	$r^2$	P	n
Control	0	3.120	(0.913)	0.709	(0.116)	0.95	0.026	4
Seasonal	30	3.490	(1.594)	0.643	(0.201)	0.72	0.033	6
Main	90	5.015	(2.901)	0.462	(0.350)	0.15	0.216	12
Combined		4.153	(0.832)	0.567	(0.104)	0.60	0.000	22

$a_0$  : intercept.

$a_1$  : slope.

(SE) : standard error.

P : significance of equation.

n : number of trees used in equation.

The slope ( $a_1$ ) is less than one, i.e. root growth shows a proportionately greater increase than the shoot. This trend is accentuated with a higher nitrogen supply, supporting the data in Table 3.12. The non significance of the regression for the 90 g N treatment is consistent with the earlier co-variance analysis (Figure 3.8). Given this it is not surprising that the regression

equations are statistically the same.

The conclusion is that root growth was proportionately greater than shoot growth on all experimental trees. The increase in root:shoot ratio with nitrogen was due in part to a natural change in allometry. The evidence for a fertilizer effect *per se* was inconclusive because one equation was non significant.

There have been a few other studies showing increased root:shoot ratios after fertilization (Table 3.14). Snowdon and Waring (1985) recognised a possible allometric effect where there was a large response to fertilizer. However, where tree sizes were similar they concluded that an inorganic nitrogen supply had stimulated root growth proportionately more than shoot growth; possibly as an opportunistic response to recover from transplanting shock. The possibility of a competition effect with the very close spacing (14233 stems/ha) was not addressed. However, Baskerville (1965) found that stand density did not significantly affect allometric relationships.

Table 3.14 Studies reporting increased root:shoot ratios following fertilization.

Site	Species	Age (yrs)	Fertilizer/tree g N g P		Yrs *	Root:shoot ratio	Reference
Bottle Lake N.Z.	radiata pine	3	0	-	1	0.434	This Study
			30	-	1	0.519	
			90	-	1	0.569	
Belanglo N.S.W.	radiata pine	4	0	0	3	0.167	Snowdon and Waring 1985
		4	26	11	3	0.191	
Toolara Qsld.	slash pine	2.8	0	43	2.8	0.236	Francis <i>et al.</i> 1984
			41	43	2.8	0.323	
Florida U.S.A.	slash pine	5	0	-	5	0.299	White <i>et al.</i> 1971
			10	-	5	0.351	

\*: years since fertilizer applied.

White *et al.* (1971) gave no explanation for the increase they observed, but Pritchett (1979, p.168) notes that the greatest increase in root biomass occurred where the soil rooting volume was low because of a high water table. Francis *et al.* (1984) also offered no explanation, but recognised the unusual nature of their results and went on to confirm an increase at other sites with both slash pine and Honduras caribbean pine (*Pinus caribaea* var. *hondurensis*). Interestingly this Queensland work was also on soils with a limited rooting volume due to a shallow soil over a clay subsoil.



The limitation of the analyses presented above is that they only present a point in time view of a dynamic process. Fine root biomass is reported to increase in the stand's early years and may reach a peak, coincident with canopy closure (Karizumi 1968). The functional balance between shoots and roots is normally perturbed by periodicity in the activity of shoot meristems (Cannell 1985). However, a balance is usually maintained over the longer term (Cannell and Willet 1976) because a period of shoot growth is often paralleled by equal and opposite fluctuations in root growth (Drew and Ledig 1980). It seems probable that the tree has initially responded to nitrogen by increasing its photosynthetic capacity. The increased photosynthate has been used by most tree components, but primarily below ground. In radiata pine leaf primordia develop some 6 to 12 months after being formed (Linder and Rook 1984). Some of the response to fertilizer will probably be delayed for this length of time, which may partly explain the limited response above ground in the first growing season. This is supported by the effect of initial tree size on final above ground biomass (Figure 3.8).

The balance between shoot and root temporarily favours the latter, but in subsequent growing seasons the shoot may respond further. At this stage of dynamic stand growth it seems likely the roots have responded to accelerate their utilisation of the site with subsequent benefits. This explanation would also support the contention of Santantonio and Herman (1985) that the growth of roots differs fundamentally from that of shoots by being far more opportunistic and exploitative.

3.3.3.4 Allocation of biomass within the crown of young radiata pine The relative allocation of biomass between tree components for this and similar studies is given in Table 3.15. As tree size increases there is a relative shift in allocation from foliage to stems and more particularly to branches during the open canopy stage (Madgwick *et al.* 1977). This same pattern may be induced with a large response to nitrogen fertilizer (Snowdon and Waring 1985). However, Nambiar and Fife (in press) found only small changes in allocation, although tree biomass was doubled. In general though nitrogen increases the proportion of branches, and this is also valid for older stands (Mead *et al.* 1984) and for other species, e.g. douglas fir (Brix 1981).

Radiata pine has a higher proportion of branches than many pines (Madgwick *et al.* 1977) and a further increase due to nitrogen fertilizer has important implications for management with regard to wood quality (Bevege 1984, Barker 1978). Stem malformation due to the loss of apical dominance on fertile sites (Will 1971) can be a problem although the extent was found to vary between two clones (Will and Hodgkiss 1977).

In this study the crown allocation patterns were not apparently affected by fertilizer (Table 3.16). An analysis using the allometric equation showed proportionately greater growth of crown and branches;

this being accentuated by fertilizer, but none of the differences was significant. The lack of changes in allocation was probably related to the relatively small above ground response and the high between tree variability.

Table 3.15 Comparative data for relative allocation of above ground biomass in young radiata pine.

Site	Age (yrs)	gN/ tree	Foliage -----	Branches %	Stem -----	Reference
Bottle Lake	2	0	53	18	29	This study
Chch. N.Z.	3	0	39	27	34	
	3	90	38	29	33	
Kaingaroa	2	0	50	18	32	Madgwick <i>et al.</i> 1977
N.Z.	4	0	32	30	38	
Spehrs Pltn.	4	0	37	26	37	Nambiar and Fife in press
S.A.	4	80	39	26	35	
Tumut For.	3	0	46	18	36	Forrest & Ovington 1970
N.S.W.	5	0	37	21	42	
Belanglo	4	0	52	13	35	Snowdon & Waring 1985
N.S.W.	4	26+P	38	16	46	
Rotorua	6	0	16	34	50	Will & Hodgkiss 1977
N.Z.	6	DT*	18	44	38	

\* double topsoil available to trees.

Table 3.16 The effect of nitrogen supply on ratios of stem to crown and branches.

Ratio	0	30	90	P
	-- grams N applied --			
Stem*: Crown#	0.486	0.455	0.475	0.836
Stem : Branches	1.218	1.085	1.124	0.753

\*: wood plus bark plus new stem.

#: foliage plus branches.

p: probability of treatment differences.

### 3.3.4 Foliage Growth Patterns

Individual needles have been used as the basis for expressing results to overcome the variation in the number of needles per fascicle between trees. Three parameters: needle length, needle weight and needles per fascicle are discussed in three sections: seasonal patterns, treatment differences and crown position effects.

3.3.4.1 Seasonal Patterns Figure 3.9 shows (a) needle length and (b) needle weight. Figure 3.10 shows needles per fascicle. The seasonal patterns were the same regardless of treatment.

1982/83 Foliage The length was constant at about 108 mm (Figure 3.9a). There was a weight gain of 4 mg per needle over the winter (excluding the 3 initial values from a different sampling scheme, see Section 3.2) (Figure 3.9b). This 20% increase could reflect storage of new photosynthate production and/or accumulation of carbohydrates from other tree tissues. The increase was in agreement with Rutter (1957), Smith *et al.* (1971), and was implied in Madgwick (1983a). However, Fife and Nambiar (1982, 1984), working with similar aged radiata pine, found no significant changes in needle weight after elongation was complete.

1983/84 Foliage Sampling of this foliage began in November although needles were appearing from early October. There was rapid elongation with an attendant weight increase until late March when needles ceased lengthening (Figure 3.9a). Needles in the upper crown were longer (143 mm) and heavier (37 mg) than those in the middle crown (134 mm and 29 mg per needle). A period of stability followed before a further gain in weight was noted during May and June, which is consistent with that observed for the previous year's foliage (Figure 3.9b). By September the needle weights were 45 and 35 mg for upper and middle crown positions respectively. This was heavier than that of the previous year's needles. While 1983/84 may have been a particularly good growing season, or 1982/83 a poor one, it is also possible that the difference was due to tree age.

The 1983/84 needles were considerably heavier than the 28 and 18 mg per needle reported by Fife and Nambiar (1984) in South Australia. On the basis of their reasoning for needles being heavier on a higher quality site (Fife and Nambiar 1982), it might be assumed that Bottle Lake is a more productive site than those studied in South Australia. However, as was noted in Section 3.3.3.1 the total above ground biomass is greater in South Australia.

Samples of fascicles from the upper crowns of individual trees had an average of 3.11 needles per fascicle with a range of 3.00-3.68. Middle crown fascicles averaged 3.04 needles per fascicle with a range of 2.99-3.15. The number of needles per fascicle was always higher in the upper crown on an individual tree basis. There was an apparent seasonal effect (Figure 3.10) with fewer needles per fascicle in

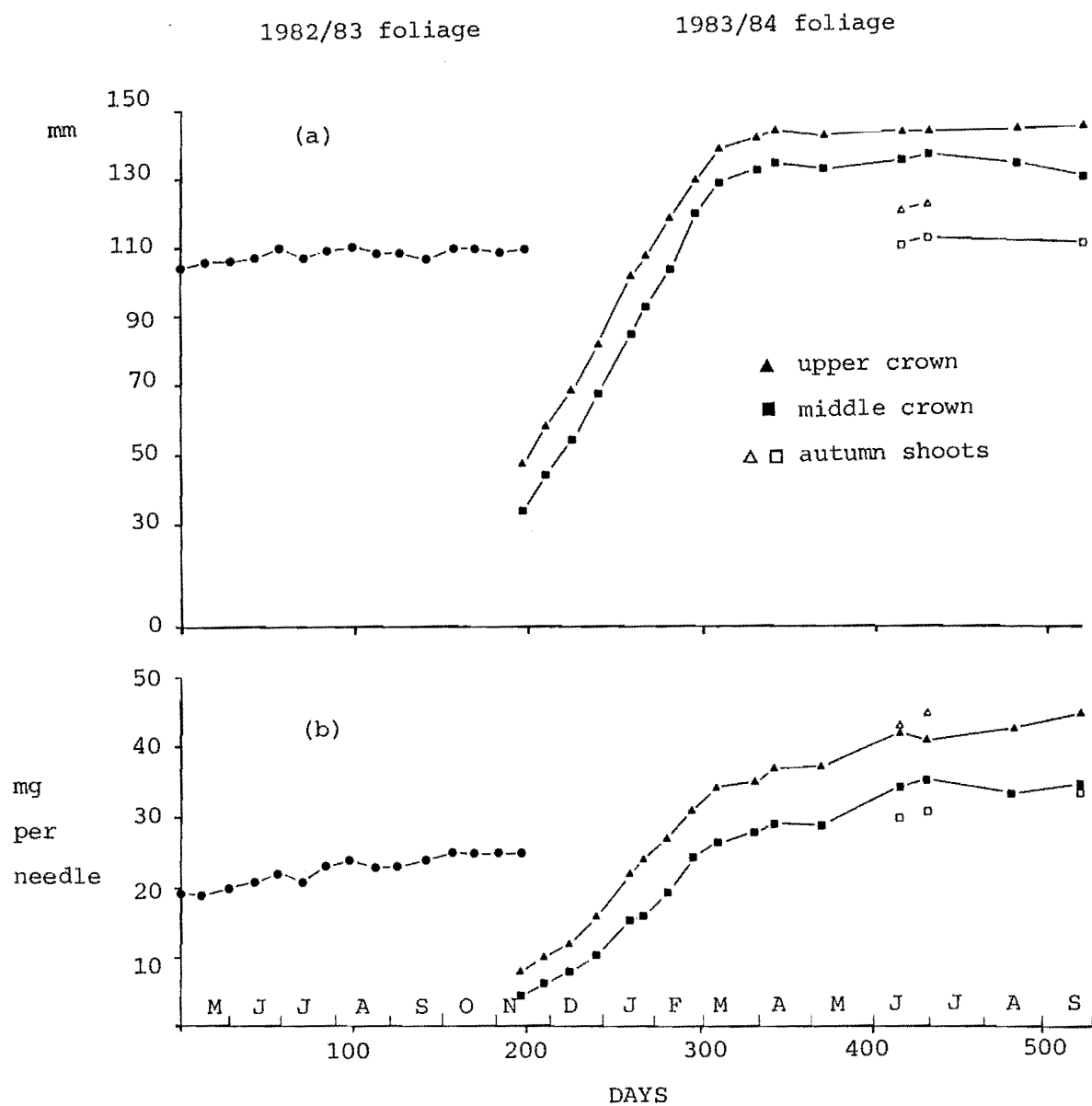
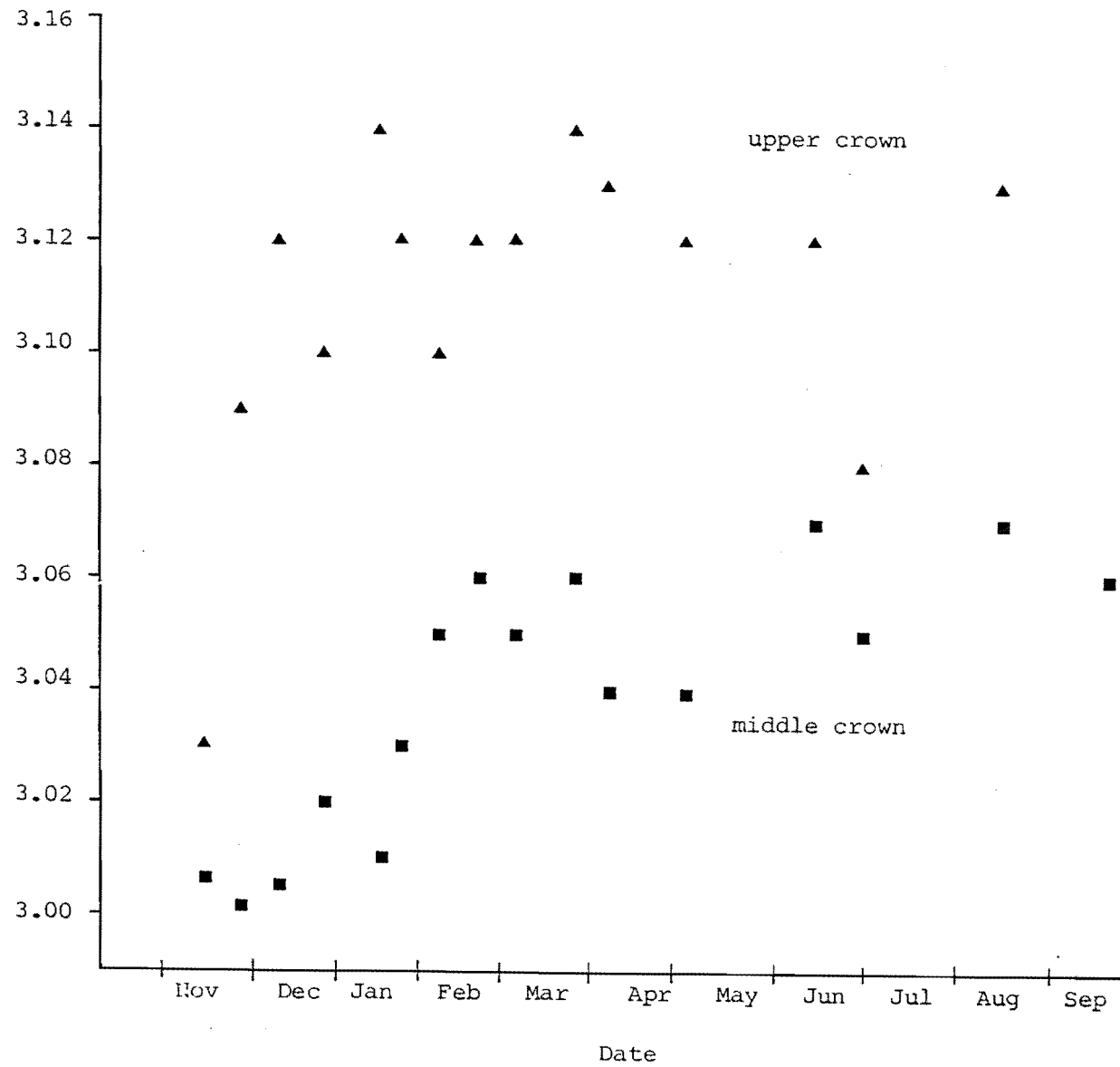


Figure 3.9 Seasonal patterns of (a) needle length and (b) needle weight

Figure 3.10

Needles per fascicle  
in the upper and  
middle crown.  
(all trees)



samples collected early in the growing season.

Towards the end of February a small but distinct flush was noted on most trees and these have been termed autumn shoots (c.f. Fife and Nambiar 1984). They occurred as extensions of the upper and middle crown branches already designated. They were sampled on three dates once elongation was complete. Autumn shoot needles in the upper crown were longer and heavier than those in the middle crown (Figure 3.9 a and b). Comparisons with the main flush were hindered because not all trees produced autumn shoots (Table 3.17). Their presence was apparently related to initial tree size, nitrogen supply and crown position. Carlson and Presig (1980) reported that nitrogen increased the proportion of douglas fir seedlings with a second growth flush.

No sectioning of the 1983/84 foliage was undertaken at the final harvest, so the proportion of autumn shoots is not known. They were, however, visually assessed to be a minor component.

Table 3.17 Percentage of trees having autumn shoots.

Treatment	Initial D <sup>2</sup> H	% trees with autumn shoots	
		in upper crown	middle crown
Control	26.48	100	50
Single	26.46	100	100
3-Split	29.08	100	100
9-Split	23.58	100	50
Autumn	19.22	50	50
Spring	26.43	100	100
Summer	23.75	50	100

3.3.4.2 Treatment Differences This section is restricted to discussing the four main treatments; Control, Single, 3-Split, and 9-Split, i.e. 16 trees.

1982/83 Foliage The weight increase during winter shown in Figure 3.9b was similar for all treatments. There were no significant differences between the weights at each date or in the % increase (Table 3.18).

1983/84 Foliage There was a 25% increase in this foliage mass for fertilized trees over Control (Table 3.6). This could be due to one of three factors or a combination of them:

- (i) increased needles per fascicle.
- (ii) increased individual needle size.
- (iii) increased total number of needles.

Table 3.18 Increase in needle weight during winter 1983.

	Control	Single	3-Split	9-Split	(SE)	P
	mg / needle					
2nd May	20	21	18.5	20.5	(1.55)	-
Oct/Nov*	25.3	25.2	24.6	24.6	(1.94)	0.700
% increase	27	20	33	20	(6.52)	0.404

\*: mean of four dates.

(i) Needles per Fascicle The effect of fertilizer on needles per fascicle was tested using analysis of co-variance with the needles per fascicle in the 1982/83 foliage as the co-variate. (This varied from 2.99-3.34 needles per fascicle with a mean of 3.09.) There was a tendency for needles per fascicle to be increased by fertilizer, but overall treatment differences were non-significant (Table 3.19). However, a single degree of freedom contrast showed that there was a significant ( $p = 0.06$ ) fertilizer effect in the upper crown.

Table 3.19 Actual needles per fascicle and adjusted means as affected by fertilizer.

Crown position	Control	Single	3-Split	9-Split	(SE)	P
	needles per fascicle*					
Upper crown (actual)	3.10	3.07	3.20	3.11	(0.086)	0.723
(adjusted)	3.04	3.14	3.17	3.13	(0.042)	0.218
Middle crown (actual)	3.04	3.02	3.05	3.06	(0.031)	0.832
(adjusted)	3.03	3.04	3.05	3.06	(0.024)	0.772

\*: mean value for all sampling dates,  $n=16$ .

(ii) Needle Weight and Length Statistical analyses were performed on needle weight data for each sampling date (15 November, 1983 - 28 September 1984). Needle length data was similarly analysed until elongation was complete (27 March, 1984). After this variability between dates is attributed solely to sampling error, so a mean length for the seven dates (27 March - 28 September, 1984) was used in the analysis.

To account for initial tree variability, an analysis of co-variance was used. For needle length the mean value of all samples from the 1982/83 foliage was used as a co-variate. For needle weight the mean of the last four sampling dates for the 1982/83 foliage was used,

as Figure 3.9b shows a stable value then. The usual requirement that the co-variate be unaffected by treatment is met in this case (Table 3.18). It is interesting to note that the increase in precision resulting from co-variance is greatest in the middle crown samples. This is presumably because these needles occurred on extensions of the branches from which the "co-variate needles" were sampled (Figure 3.1b).

Results for needle length and weight in the upper and middle crown are given in Appendix 7. The results from partitioning the ANCOVA into a single degree of freedom contrasts are summarised in Tables 3.20, 3.21, 3.22 and 3.23.

Table 3.20 Probabilities (p) of treatment differences in upper crown needle length, according to single degree of freedom contrasts.

Date		Control vs fertilized	Single vs Split P	3 vs 9 Split
November	15	0.434	0.239	0.824
November	28	0.466	0.451	0.637
December	28	0.216	0.740	0.882
January	16	0.129	0.203	0.549
January	24	0.290	0.913	0.185
February	7	0.092	0.533	0.571
February	21	0.352	0.146	0.225
March	6	0.334	0.484	0.032
Mar-Sept*		0.135	0.905	0.033

\* : data for 7 dates (Mar. 27 - Sept. 28) combined.

There was no statistically significant increase in needle length in the upper crown due to fertilizer (Table 3.20). The needles on 9-Split trees finally became longer than those on the 3-Split trees. The pattern in the middle crown was different with a fertilizer effect being apparent from December to February (Table 3.21). However, once elongation was complete fertilized needles were not significantly longer. The difference between 3 and 9-Split trees was again apparent.



Table 3.21 Probabilities (p) of treatment differences in middle crown needle length, according to single degree of freedom contrasts.

Date		Control vs fertilized	Single vs Split	3 vs 9 Split
		P		
November	15	0.285	0.063	0.683
November	28	0.205	0.288	0.947
December	13	0.074	0.151	0.969
December	28	0.080	0.299	0.897
January	16	0.029	0.551	0.454
January	24	0.013	0.239	0.765
February	7	0.006	0.782	0.889
February	21	0.108	0.972	0.488
March	6	0.466	0.723	0.314
Mar-Sept*		0.481	0.950	0.024

\* : combined data for 7 dates (Mar. 27 - Sept. 28).

Table 3.22 Probabilities (p) of treatment differences in upper crown needle weight, according to single degree of freedom contrasts.

Date		Control vs fertilized	Single vs Split	3 vs 9-Split
		P		
November	15	0.168	0.570	0.673
November	28	0.224	0.616	0.480
December	28	0.118	0.932	0.674
January	16	0.063	0.613	0.264
January	24	0.080	0.935	0.141
February	7	0.018	0.948	0.221
February	21	0.006	0.540	0.071
March	6	0.012	0.407	0.024
March	27	0.048	0.778	0.001
April	7	0.052	0.665	0.084
May	5	0.076	0.932	0.060
June	20	0.027	0.709	0.039
July	5	0.162	0.894	0.092
August	21	0.076	0.427	0.057
September	28	0.121	0.829	0.035

Table 3.23 Probabilities (p) of treatment differences in middle crown needle weight, according to single degree of freedom contrasts.

Date		Control vs fertilized	Single vs split	3 vs 9 split
		P		
November	15	0.388	0.521	0.337
November	28	0.269	0.425	0.648
December	13	0.286	0.402	0.848
December	28	0.139	0.459	0.627
January	16	0.054	0.726	0.240
January	24	0.036	0.152	0.390
February	7	0.003	0.610	0.271
February	21	0.058	0.513	0.304
March	6	0.147	0.507	0.099
March	27	0.011	0.193	0.590
April	7	0.076	0.445	0.054
May	5	0.049	0.448	0.125
June	20	0.288	0.850	0.086
July	5	0.229	0.784	0.004
August	21	0.191	0.990	0.186
September	28	0.072	0.743	0.071

Needle weight in the upper crown of fertilized trees was significantly heavier by January (Table 3.22). From March the difference between 3 and 9-Split was also statistically significant. A similar trend was also apparent for the middle crown needles (Table 3.23) although the difference between split treatments was not so pronounced.

There was an indication of more rapid growth of needles on fertilized trees, but finally there were no statistically significant differences. This was partly due to the difference between fertilizer treatments. The 3-Split needles were either the same size or smaller than Controls. The 9-Split needles, however, were 5% longer and 32% heavier than Controls in the middle crown by the end of the experiment (Appendix 7).

(iii) Number of Needles This was not assessed at the final harvest but an estimate was calculated for the 1983/84 (1-year) foliage as an indication of the response to fertilizer. The mass of 1-year needles was divided by the best approximation of individual needle weight, i.e. an average of upper and middle crown needle weight for September 28 (11 days before the final harvest) (Table 3.24). There appears to be an inverse relationship between foliage mass and

individual needle weight for the fertilized trees. The Single and 3-Split treatments have responded by producing more needles. There are apparently fewer needles on the 9-Split trees.

Table 3.24 1983/84 foliage mass, needle weight and needle number.

	Control	Single	3-Split	9-Split
Foliage mass (g)	1571	2002	2196	1696
Needle weight (mg)	36.5	41.5	37.5	45.5
Number of needles	43,000	48,000	59,000	37,000

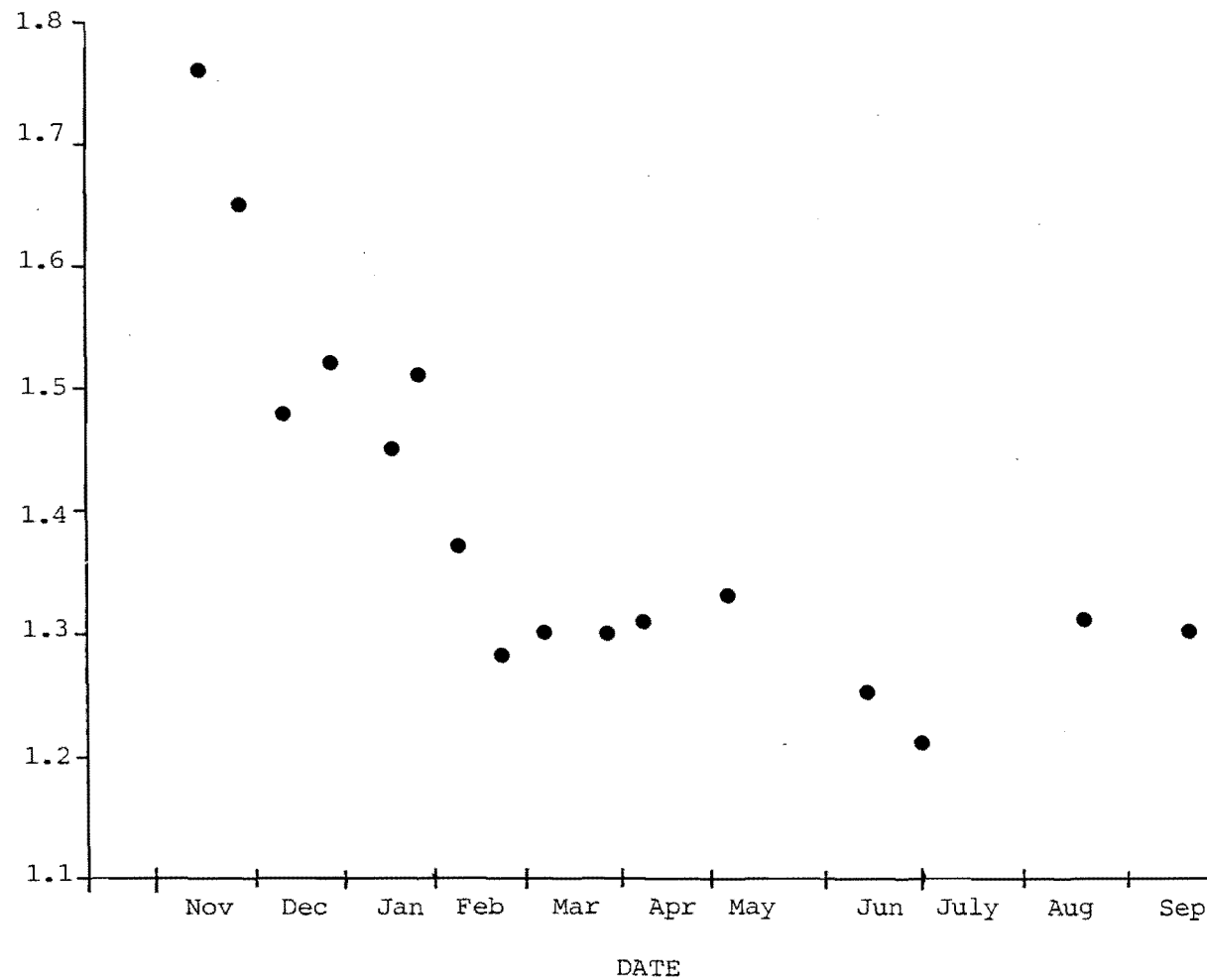
The three processes which could have produced the 25% weight increase in foliage following fertilization seem to have acted differently across treatments. The Single treatment has responded with more and heavier needles. The 3-Split shows no increase in needle weight by the end of the experiment, but has many more needles. This is partly due to the greater number of needles per fascicle (Table 3.19). It is not suggested that these differences are due to the method of fertilizer application, but probably to initial tree size. The weight of older foliage (which is an estimate of initial foliage mass) was 561, 602, 688 and 435 grams for the Control, Single, 3-Split and 9-Split treatments respectively. It is suggested that the larger 3-Split trees had a greater capacity for initiating more short shoots (fascicles), whereas the small 9-Split trees were restricted in this aspect and developed heavier individual needles. This is further supported by the growth of autumn shoots which were especially pronounced on the 3-Split trees but absent from some of the 9-Split trees (Table 3.17).

3.3.4.3 Crown Position Differences It has already been noted that upper crown needles were longer and heavier than those in the middle crown. The number of needles per fascicle was also greater in the upper crown. There is some evidence for fertilizer having a greater effect on middle crown needles, e.g. a greater percentage of weight increase than in the upper crown (Appendix 7), and significantly longer needles (Table 3.21). This would be in agreement with the hypothesis that fertilized trees increase their foliage mass by becoming more "bushy" with expansion in the middle crown (Will and Hodgkiss 1977). An analysis was run on the needle weight ratios between upper and middle crown. There were no significant differences between treatments indicating that a reasonable balance was maintained within the crown regardless of the nitrogen status on this site. The analysis does, however, show a seasonal trend in the ratio (Figure 3.11). Initially upper crown needles were much heavier, but this declined to about a 30%

Figure 3.11

Ratio of upper :

middle crown  
individual  
needle weight



increase over middle crown needles. This pattern could be explained if upper crown needles were initiated earlier. It may also be regarded as showing the early season concentration of growth in the upper crown followed by a diversion of relatively more resources to the middle crown as the season progresses.

### 3.4 CONCLUSION

Applications of 90 g N per tree, whether applied singly or as split dressings, resulted in a 30% biomass response. This was particularly pronounced below ground, which was related to the exploitative nature of the roots and the limits on above ground production in the first year after fertilization. The 25% increase in foliage formed during the first year of response was attributed to an increase in needle weight, number of needles (e.g. autumn shoots) and, partly, number of needles per fascicle.

Applications of 30 g N per tree gave a below ground response, but no statistically significant increase above ground.

The foliage elongated from October 1983 until March 1984. Needle weight then stabilised, which coincided with the formation of autumn shoots on most trees. After these were formed and when stem diameter growth ceased in May, there was a further increase in needle weight. The seasonal growth of rootstock and coarse roots probably paralleled stem growth (NZFS, 1985). The timing of fine root growth and in particular the large response on fertilized trees is more equivocal. If major growth phases of root and shoot are episodic, as reported for loblolly pine seedlings (Drew and Ledig 1980), it is probable that the majority of the fine root response occurred after the main foliage flush, i.e. from March onwards.

Had this study relied on above ground biomass determinations, and an assessment of stem diameter at 15 cm, the effect of fertilizer might have seemed minor. Doubts would have been raised about the benefits of nitrogen fertilization on sandy soils (c.f. Nambiar and Cellier 1985). However, this study shows that the major response to nitrogen in young pines may be hidden below ground. This should confer stability and, in subsequent years, a greater ability to absorb water and nutrients, and produce extra growth. An appropriate step to detect this below ground response may be to measure diameter at the base of the stem.

## CHAPTER 4

## FOLIAR NUTRIENTS : UPTAKE AND PARTITIONING

## 4.1 INTRODUCTION

Foliar nutrient concentrations (grams element per gram tissue) and nutrient content (grams element per fascicle or needle) are used to monitor the nutritional status of pine forests (Will 1985), to detect treatment differences (Mead 1984) and to investigate nutrient retranslocation (Fife and Nambiar 1982, 1984). Several reviews of foliar analysis as a tool in nutritional research are available (Mead 1984, Lambert 1985 and van den Driessche 1974). A further detailed review is not appropriate here. This chapter concentrates on the effect of nitrogen fertilizer on the seasonal patterns of foliar nitrogen and its partitioning within the crown. The use of foliar nitrogen analysis to monitor the uptake of fertilizer nitrogen into the tree is presented in Chapter 5. Nitrogen should not be studied in isolation as it is linked with other elements in physiological processes. Accordingly the levels of other nutrients and their interactions with nitrogen are briefly discussed.

## 4.2 METHODS

The collection, drying and weighing of foliage samples have been described in Chapter 3. After drying, samples were finely ground in a Rocklabs Mill. Care was taken to prevent cross contamination by careful cleaning between samples, and by ensuring that unfertilized tree samples were ground first in a batch.

Samples collected on at least 0, 14, 55 and 110 days after each major fertilizer application (May, August and December 1983) were analysed for total nitrogen. Additional samples were analysed to characterise more clearly the seasonal patterns on this site. All trees were analysed individually. Only data from the main treatments are discussed, except where the seasonal treatments provide additional information.

4.2.1 Total Nitrogen

A modified semi-micro Kjeldahl digestion procedure was used, adapted from Bremner and Mulvaney (1982), Buresh *et al.* (1982), Nelson and Sommers (1980) and Nicholson (1984). Finely ground 200 mg samples were digested with one sodium sulphate/selenium catalyst tablet (containing 1 g  $\text{Na}_2\text{SO}_4$  and 0.05 g Se) and 4 ml concentrated sulphuric acid in a Tecator aluminium digestion block. The block was preheated to

200°C and increased to 375°C during digestion. The samples took 50 minutes to "clear", after which the temperature was maintained for 2 hours. Digests were then cooled and diluted to 25 ml.

Nitrogen was determined in aliquots of digests, by steam distillation in the presence of sodium hydroxide. Ammonia was absorbed in boric acid indicator solution and titrated with standardised 0.01 N sulphuric acid. Two steam distillation units were constructed (c.f. Keeney and Nelson 1982, p.652, and Greenfield pers. comm.) and run alternately from the same steam source. The results were converted to an oven dry basis using a moisture factor, determined by drying a portion of the original ground sample at 65°C for 24 hours. Corrections were also made for the efficiency of the steam distillation units by distilling aliquots of a standard ammonium sulphate solution. The recovery was usually > 98%.

The method was rigorously checked by digesting various nitrogen compounds, to determine recoveries, and by participating in the 1984 International Union of Forestry Research Organisations (IUFRO) interlaboratory comparison for foliar analysis. The two foliage samples (84/1 radiata pine and 84/2 *Eucalyptus nitens*) were distributed by Dr.G.M. Will who reports the results in Will (1986). This method was compared with that of Parkinson and Allen (1975), modified by Nicholson (1984) (Table 4.1).

Table 4.1 Results from 1984 interlaboratory comparison and recoveries of two nitrogen compounds.

Method	IUFRO foliage samples*		EDTA	Glycine
	84/1	84/2	-- % recovery --	
	-- % oven dry weight --			
Na <sub>2</sub> SO <sub>4</sub> / Se#	1.31	1.88	98	98
H <sub>2</sub> O <sub>2</sub> / H <sub>2</sub> SO <sub>4</sub> +	1.27	1.80	80	92

\*: international means with (s.d.) were 84/1: 1.30 (0.09)  
84/2: 1.81 (0.17).

#: this study.

+: Parkinson and Allen (1975).

Samples were analysed in duplicate and the usual difference relative to their mean was 1-3%. Differences > 4% were rejected and the analysis repeated.

#### 4.2.2 Other Nutrients

Analyses were performed for a number of elements, using an X-ray fluorescence (XRF) spectrophotometer (Jones 1982, Norrish and Hutton

1977). Concentrations of elements in the sample were calculated from comparison with a calibration curve. The results are only as accurate as the standards used in preparing the calibration. In this study the standards used are six plant materials from the Commonwealth, Scientific and Industrial Research Organisation (analysed by XRF), three from the National Bureau of Standards (analysed by chemical methods), and three cellulose blanks. Regressions were run for each element and any standard lying  $> 2$  s.d. from the calculated value was rejected from the calibration.

Sample preparation consisted of making a firm pellet from finely ground needles in an Analytron Auto Press. Polyvinyl acetate solution was used to ensure a robust pellet. Because of the limited sample size the analyses were on a bulked treatment basis.

The foliage collected at the final biomass (Chapter 3) was also analysed by XRF. In this case individual trees were able to be analysed separately.

#### 4.3 RESULTS AND DISCUSSION

##### 4.3.1 Nitrogen Concentration

The foliar nitrogen concentrations are shown for the main treatments only (Figure 4.1). Data for the mature 1982/83 foliage are shown until sampling is changed to the newly expanding 1983/84 foliage. Only data for the upper crown needles are presented here. Differences between crown positions are discussed in Section 4.3.2. The following sections, 4.3.1.1 - 4.3.1.4, all refer to Figure 4.1.

4.3.1.1 1982/83 Foliage - Unfertilized The data for all unfertilized trees during the winter (May - August, 1983) show a slight rise from 1.61 - 1.66% N. A pronounced rise was apparent on Control trees in spring, reaching 1.85% N by the beginning of November (Figure 4.1). This rise may be attributed to uptake of soil nitrogen or retranslocation from other tree components. The decline from mid November was also apparent on unfertilized Summer treatment trees (data not presented).

4.3.1.2 1982/83 Foliage - Fertilized Fertilizer applications increased nitrogen concentrations markedly in these mature needles; however, the same decline was apparent in November. The 9-Split treatment appeared to elicit a larger response in foliar nitrogen concentration (Figure 4.1). This should not necessarily be used to indicate greater uptake of fertilizer, as factors other than nitrogen supply will influence tissue concentration. Of these, needle size, the number of needles per tree, and initial nitrogen concentration may be important. Co-variance analysis using initial foliar nitrogen concentration as the co-variate had little effect on the values (Table



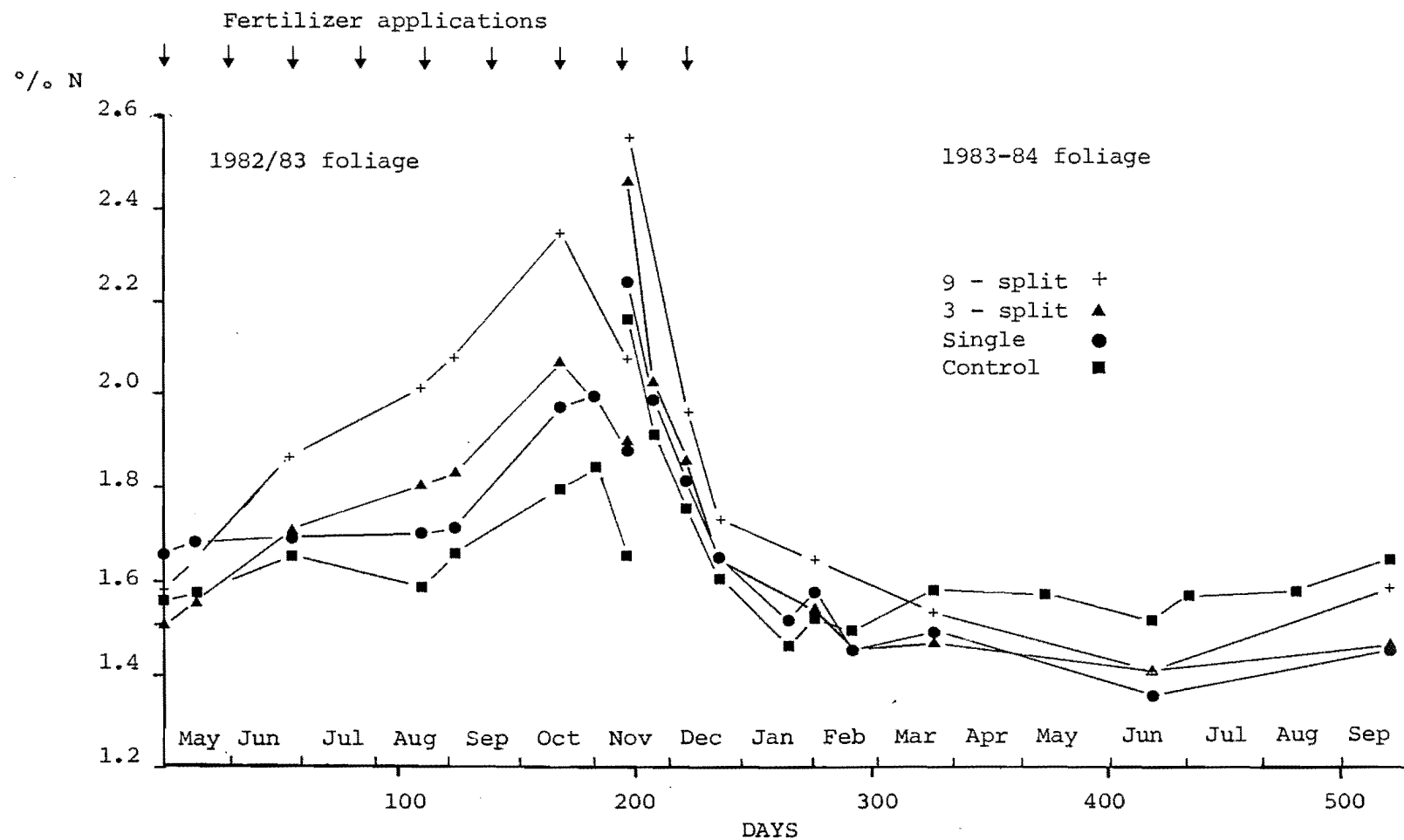


Figure 4.1 Foliar nitrogen concentration in upper crown needles

4.2). The reciprocal of older foliage biomass (Chapter 3) was also tried as a co-variate (Table 4.3). Foliage biomass, which is an indicator of the total number of needles because of the similar needle weights (Table 3.18), was considered critical because if there were a smaller number of needles, each one would receive more nitrogen, resulting in a higher concentration, *ceterus paribus*.

Table 4.2 Nitrogen concentration in 1982/83 foliage on 22 August, 1983, with means adjusted for initial nitrogen concentration.

	Control	Single	3-Split	9-Split	(SE)
	----- % N oven dry weight -----				
Actual	1.59	1.70	1.81	2.02	(0.069)
Adjusted	1.60	1.66	1.84	2.02	(0.069)

(SE): standard error.

Table 4.3 Nitrogen concentrations in 1982/83 foliage with means from analysis of co-variance with old foliage biomass.

Date	Control	Single	3-Split	9-Split	(SE)	P
	----- % N oven dry weight -----					
May 2						
actual	1.56	1.66	1.51	1.59	(0.043)	0.154
adjusted	1.57	1.67	1.54	1.55	(0.043)	0.152
August 22						
actual	1.59	1.71	1.81	2.02	(0.069)	0.005
adjusted	1.60	1.73	1.87	1.92	(0.060)	0.019
November 15						
actual	1.66	1.88	1.89	2.08	(0.081)	0.025
adjusted	1.67	1.90	1.95	1.99	(0.077)	0.066

(SE): standard error.

p : probability of treatment differences according to ANOVA and ANCOVA respectively.

Analyses of the probabilities in Table 4.3 with single degree of freedom contrasts showed that the significant increase was above Controls and not between fertilizer treatments. The "superiority" of

the 9-Split treatment is apparent, not real, and due to inherent tree characteristics.

4.3.1.3 1983/84 foliage - Control The young needles initially had high concentrations of nitrogen, which declined rapidly as carbon fixation exceeded nitrogen accumulation. In New Zealand the foliar nitrogen status is preferably assessed from January to March (Mead and Will 1976) when trees are under maximum stress. The minimum value in late January was 1.47% N which is borderline between the marginal and satisfactory categories given for radiata pine (Will 1985). It should be noted that these are young trees and critical levels are thought to vary with the developmental stage of trees (Miller *et al.* 1981). Lambert (1985) shows that nitrogen concentration falls from 1.8 to 1.1% in radiata pine from age 1 - 7 years. Nambiar and Bowen (1986) report that nitrogen concentration has decreased from 2.03% in 1-year-old trees to 1.33% in 4-year-old trees. Because critical levels are generally poorly defined (Mead 1984) and because of the growth response to nitrogen (Chapter 3), it is assumed that this site is marginally deficient in nitrogen.

Miller *et al.* (1981) found that optimum height growth for corsican pine occurred at a lower nitrogen concentration than for diameter or volume increment. It is likely that the 1.47% N reported above is at least optimal for height growth of radiata pine, given the lack of response in this parameter. The diameter response indicates that 1.47% N is less than optimal for radial growth (Chapter 3).

After the summer minimum the concentration tends to rise through the remainder of the growing season and into the next one. The value in August, 1984 (1.6% N) is the same as that in August 1983, although needles were 78 % heavier and total tree biomass had increased by about 600% (Chapter 3). Clearly the decline in nitrogen concentration with age, reported above, has not yet occurred. The nitrogen supply must be reasonable to maintain concentration with such a large biomass increase. Lambert (1985) argues for a temporary rapid mineralization rate associated with disturbance at planting.

The seasonal patterns were in general agreement with Fife and Nambiar (1982) working with similar aged radiata pine in South Australia. However, their minimum value (in March) was 1.1% N on trees which had received nitrogen at planting and one year later.

4.3.1.4 1983/84 Foliage - Fertilized Trees The foliar nitrogen levels were initially very high, up to 2.7% N. These upper crown needles had significantly higher values than Controls (Table 4.4). However, the concentration declined rapidly as needles expanded and within 6 weeks they were not significantly higher than Controls. This decline occurred regardless of two treatments being fertilized on December 13. In contrast to the Control trees, concentrations continued to fall beyond the usual summer minimum and eventually became significantly lower (Table 4.4). The minimum concentrations in June

were 1.37, 1.43 and 1.42% N for the Single, 3-Split and 9-Split treatments respectively. These levels are considered marginal, although their actual effect on photosynthesis is unknown (Fife and Nambiar 1982).

The decline in late summer and autumn could be a dilution effect due to additional growth induced by fertilizer (see review by Jarrell and Beverly 1981). Alternatively it could be a result of retranslocation of nitrogen from these needles to autumn shoot growth or root production. This aspect is discussed later in Section 4.3.2 on nitrogen content, where the incorporation of needle weight data allows more definite statements on retranslocation.

Table 4.4 Mean nitrogen concentrations in upper and middle crown needles of 1983/84 foliage as affected by fertilizer.

Date		Control	Single	3-Split	9-Split	(SE)	P
		----- % N oven dry wt -----					
November 15	Upper	2.17	2.25	2.46	2.55	(0.091)	0.034
	Middle	1.89*	1.93	1.98	2.02	(0.073)	0.358
December 13	Upper	1.76	1.88*	1.85	1.97	(0.055)	0.050
	Middle	1.63	1.76	1.69	1.79	(0.057)	0.089
December 28	Upper	1.61	1.66	1.66	1.74	(0.050)	0.241
	Middle	1.50	1.67	1.63	1.69	(0.053)	0.020
February 7	Upper	1.53	1.58	1.55	1.65	(0.053)	0.283
	Middle	1.46	1.56	1.55	1.61	(0.051)	0.093
March 27	Upper	1.57	1.50	1.48	1.54	(0.063)	0.395
	Middle	1.45	1.47	1.46	1.57	(0.054)	0.492
June 20	Upper	1.53	1.37	1.43	1.42	(0.037)	0.014
	Middle	1.44	1.33	1.38	1.41	(0.050)	0.252
September 28	Upper	1.66	1.47	1.48	1.60	(0.057)	0.051
	Middle	1.48	1.37	1.39	1.54	(0.046)	0.438

\* : three replicates only.

(SE): standard error.

p : probability of differences between Control and fertilized trees according to single degree of freedom contrast from ANOVA.

The general trends for middle crown needles (data not presented graphically) are similar. The absolute values tend to be lower (see

Section 4.3.3) with the fertilized trees reaching a minimum of 1.33, 1.37 and 1.41% N in June for the Single, 3-Split and 9-Split treatments respectively. Concentrations in the middle crown are not initially significantly higher than Controls (Table 4.4), but become so when the upper crown differences disappear. The nitrogen concentration in middle crown needles on fertilized trees did not become significantly lower than Controls (Table 4.4).

#### 4.3.2 Nitrogen Concentration Gradients

Foliar nitrogen concentrations often vary with position in the crown for a given age of foliage (Mead 1984). It is widely assumed that nitrogen concentration decreases from the top to the base of the crown (Madgwick *et al.* 1983), although van den Driessche (1974) gives examples of five different gradient patterns. Gradients within the crown may be better than single critical values for investigating nutrient status, although Mead (1984) cautions against confounding the interpretation with variable light conditions.

A decrease in nitrogen concentration down the crown has recently been reported for two stands of radiata pine: at the age of 7 (Madgwick *et al.* 1983) and at the age of 10 (Cromer *et al.* 1985). However, Fife and Nambiar (1982) found no significant differences between primary and secondary branch needles in 3-year-old radiata pine. Their sampling positions correspond roughly with the upper and middle crown positions designated in this study.

In this study the effect of nitrogen supply and seasonal patterns on the gradients within the crown were investigated. The statistical analyses were performed on the difference in nitrogen concentration between upper and middle crown needles on an individual tree basis. This removes the problem of variability between trees when absolute values are used.

On an individual tree basis the nitrogen concentration in Controls was always higher in the upper crown (except for one tree on June 20). This trend was also apparent initially for the fertilized trees, but in later samplings the difference could be either positive or negative. Accordingly the results have been presented as either the absolute value difference between upper and middle crown (Table 4.5) or the difference (upper - middle) (Table 4.6) which includes some negative values.

**4.3.2.1 Seasonal Trends** In very young needles the gradients within crowns were quite large, which reflected the actual nitrogen concentrations (Figure 4.1, Table 4.4). The differences decreased but later in the season stabilised at about 0.1% N in the Controls. For all treatments, the gradient increased in the following spring (Table 4.6).

It appears that early in the growing season there was a preferential allocation of nitrogen to the apical region. This may

relate to the hormonal control from this area (van den Driessche 1974). This trend relates well with the growth of needles (Chapter 3, Figure 3.11). As the season progressed a relative shift to growth in the middle crown needles occurred and the smaller nitrogen gradients reflected this.

Table 4.5 Absolute value difference in %N between upper and middle crown needles.

Date	Control	Single	3-Split	9-Split	P
November 15	0.28	0.33	0.48	0.53	0.105
December 13	0.13	0.12	0.15	0.18	0.660
December 28	0.11	0.05	0.06	0.04	0.004
February 7	0.07	0.05	0.03	0.07	0.470
March 27	0.12	0.03	0.06	0.04	0.070
June 20	0.10	0.09	0.11	0.10	0.964
September 28	0.18	0.09	0.08	0.06	0.016

p : probability of differences between control and fertilized trees according to single degree of freedom contrast in ANOVA.

Table 4.6 Difference in %N : (upper - middle) crown needles.

Date	Control	Single	3-Split	9-Split	P
November 15	0.28	0.33	0.48	0.53	0.105
December 13	0.13	0.12	0.15	0.18	0.660
December 28	0.11	-0.01	0.02	0.04	0.011
February 7	0.07	0.03	0.01	0.05	0.255
March 27	0.12	0.03	0.02	-0.03	0.025
June 20	0.09	0.03	0.05	0.01	0.425
September 28	0.18	0.09	0.08	0.06	0.016

p : probability of differences between control and fertilized trees according to single degree of freedom contrast in ANOVA.

4.3.2.2 Treatment Differences Gradients were maintained in Control trees but were virtually absent from fertilized trees (Table 4.6). This was in part due to positive and negative differences, cancelling each other out. However, the absolute value differences

(Table 4.5) also show that gradients were generally smaller on fertilized trees by late December.

Cromer *et al.* (1985) argued that in a severely phosphorus deficient stand (foliar P = 0.07%) there could be no gradients within the crown, presumably because this is the minimum concentration required to maintain basic metabolic functions. When this deficiency was partially alleviated by fertilizer (P = 0.12%), gradients appeared within the crown, with preferential allocation to the apical regions. If nitrogen allocation patterns are similar to those of phosphorus, then gradients should decrease with lower nitrogen concentrations. High initial concentrations (Figure 4.1) equate with large gradients (Table 4.5). Lower concentrations on fertilized trees later in the season seem to correspond with smaller or non existent gradients. This correlation was tested for all trees (Table 4.7). The hypothesis was generally valid, especially early in the growing season when gradients were more pronounced.

Table 4.7 Correlations between the %N difference (Upper - Middle) and %N in upper crown.

Date	n	r	P
November 15	21	0.812	0.000
December 13	21	0.437	0.024
December 28	22	0.156	0.243
February 7	22	0.279	0.104
March 27	22	0.507	0.008
June 20	22	0.309	0.081
September 28	16	0.594	0.007

n : number of trees.

r : correlation co-efficient.

P : significance of correlation

The effect of fertilizer has been to disrupt the gradient pattern within the crown in which the upper crown predominates. Gradients are smaller on fertilized trees which accords with their lower nitrogen concentration. If it is argued that stress leads to smaller gradients, then an induced nitrogen deficiency is apparent on fertilized trees. This might be assumed from the absolute nitrogen levels (1.4% N). This may seem somewhat incongruous, given that the trees have just been fertilized. However, if the decline in nitrogen concentration with age (Lambert 1985) is applicable at Bottle Lake, then the effect of fertilizer could be to accelerate the trees along a natural decline in concentration. The erratic gradients on fertilized trees suggest that a limited nitrogen supply is being distributed within the crown to where maximum carbon gain is possible (c.f. Field 1983).

Madgwick *et al.* (1983) found a difference of 0.06% N between needles in the "top quarter" and "upper middle" crowns of 7-year-old radiata pine. This is slightly lower than the gradient at Bottle Lake which agrees with the slightly lower concentrations in their study. However, no differences between nutritional treatments (including urea application) were apparent. The foliage sampling was confounded by time and a recent application of fertilizer. Given the importance of seasonal trends and response to fertilizer found in this study, the non significant results of Madgwick *et al.* (1983) are hardly surprising.

In discussing gradients, and by implication variable demands for nitrogen within the tree, it should be noted that total nitrogen status is a rather crude parameter to use. It is likely that some measure of the mobile nitrogen pool, exclusive of structurally bound nitrogen (c.f. Fagerström and Lohm 1977, Miller *et al.* 1979), would be more useful in this analysis.

#### 4.3.3 Needle Nitrogen Content

Fife and Nambiar (1982, 1984) stress the importance of expressing foliar nitrogen on a per needle basis to investigate translocation within the crown. Accordingly nitrogen content has been expressed as  $\mu\text{g N}$  per needle, the product of needle weight (Chapter 3), and nitrogen concentration (Section 4.3.1). The results are shown for the main treatments in Figure 4.2. Increases represent net accumulation of nitrogen, and declines represent net retranslocation.

4.3.3.1 1982/83 Foliage The nitrogen content in the mature 1982/83 needles (Figure 4.2) largely reflected the nitrogen concentration patterns (Figure 4.1). The decline in November indicated movement of nitrogen from these needles to other parts of the tree, presumably the newly expanding foliage. The only other explanation for this decrease would be foliar leaching. However, leaching of nitrogen is reported to be very low (Tukey 1970), canopy nitrogen being tightly conserved (Parker 1983), and net absorption from aerosol inputs possible (Baker *et al.* 1985). Furthermore only 5.5 mm of rain fell in the first half of November (Figure 2.1).

4.3.3.2 1983/84 Foliage - Seasonal Patterns As the new foliage expands (Figure 3.9) there was a rapid accumulation of nitrogen (Figure 4.2). This slowed down once needle elongation was complete, but continued throughout the remainder of the season and into the next one for the upper crown needles. The pattern for the middle crown needles is similar except net accumulation ceased later in the season for three of the treatments. These trends contrast with those reported for young radiata pine in South Australia (Fife and Nambiar 1982, 1984), which show phases of accumulation and withdrawal of nitrogen from needles < 1-year-old. It has already been noted (Chapter 3) that needle weight is much less, and total above ground biomass greater in South



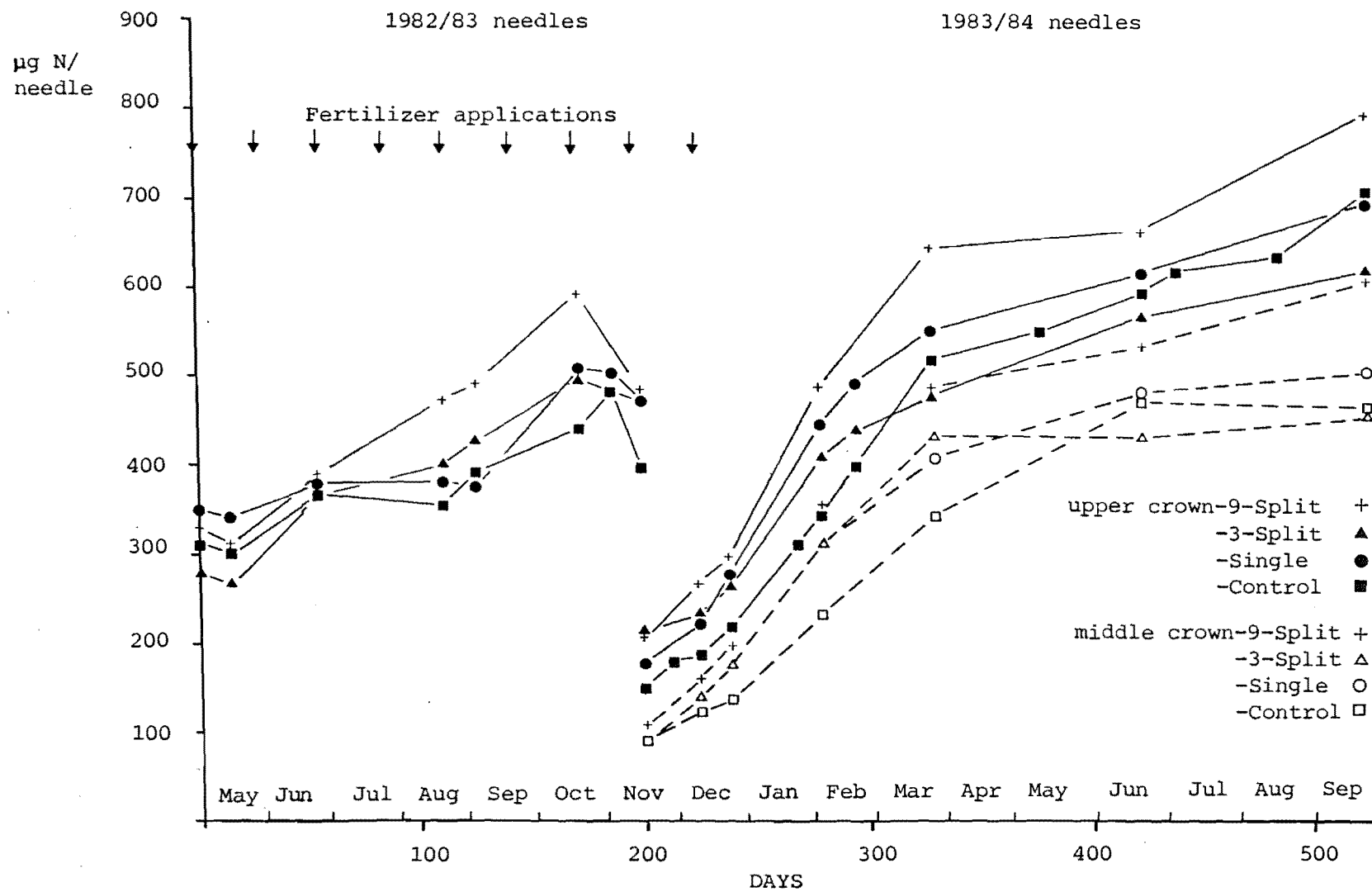


Figure 4.2 Foliar nitrogen content

Australia. The demands of growth are apparently greater there, leading to lower nitrogen concentrations (1.1%) and no gradients within the crown.

4.3.3.3 1983/84 Foliage - Treatment Differences Fertilized trees initially had significantly higher contents in the upper crown needles, which was apparent until February (Table 4.8). Differences then decreased as the Controls accumulated nitrogen more rapidly (Figure 4.2). The 3-Split trees had lower and the 9-Split trees higher nitrogen contents in accordance with their different needle weights (Chapter 3).

Table 4.8 Mean nitrogen content in upper and middle crown needles of 1983/84 foliage as affected by fertilizer.

Date			Control	Single	3-Split	9-Split	(SE)	P
			ug N/needle					
November	15	Upper	151	179	215	208	(17.4)	0.028
		Middle	88a	86	89	107	(10.9)	0.677
December	13	Upper	183	214a	231	269	(26.5)	0.100
		Middle	122	141	148	161	(15.9)	0.147
December	28	Upper	219	277	269	298	(25.1)	0.051
		Middle	137	175	180	198	(16.2)	0.027
February	7	Upper	346	446	411	487	(21.5)	0.002
		Middle	233	321	315	356	(22.3)	0.003
March	27	Upper	520	551	477	643	(23.7)	0.204
		Middle	345	410	434	486	(37.0)	0.039
June	20	Upper	597	615	569	665	(35.0)	0.641
		Middle	473	481	433	538	(43.6)	0.831
September	28	Upper	709	697	624	798	(56.7)	0.973
		Middle	467	507	466	626	(55.0)	0.322

a : three replicates only.

(SE): standard error.

p : probability of differences between Control and fertilized trees according to single degree of freedom contrast from ANOVA.

Significantly higher contents in the middle crown fertilized needles were not apparent until December 28 (Table 4.8). Differences

were maintained longer than in the upper crown but the same pattern eventuated. The higher values for the 9-Split trees may again be primarily explained by the heavier needles in this treatment (Chapter 3).

There was no net retranslocation of nitrogen from the 1983/84 needles during their first year after initiation. However, the relatively greater accumulation of nitrogen in Controls suggested that nitrogen was being directed to other tissues on fertilized trees. The presence of autumn shoots was more apparent on fertilized trees which would have been a sink for nitrogen (c.f. Fife and Nambiar 1984). However, the main difference between Control and fertilized trees was the increase in below ground biomass. This has probably utilised large amounts of nitrogen which would otherwise have accumulated in the needles. The slowing of foliar nitrogen accumulation on fertilized trees later in the season might suggest that this was when a major alternative sink was forming, e.g. the fine root response.

#### 4.3.4 Other Nutrients

The results for the IUFRO standards are given in Appendix 8. With the exception of potassium (which was overestimated by 10%), the agreement with the international means was good and enables comparisons to be made with some confidence. The concentrations of all elements from the XRF analysis were similar across treatments at the beginning of the experiment (Table 4.9). The exception was the 3-Split treatment with higher values for some elements, notably potassium and magnesium.

Table 4.9 Elemental concentrations in 1.7-year-old radiata pine at the beginning of the experiment (May, 1983).

Treatment	Element							
	P	K	Ca	Mg	Si	Cl	S	Al
	----- % oven dry weight -----							
Control	0.16	0.85	0.29	0.13	0.04	0.18	0.11	0.05
Single	0.16	0.85	0.27	0.13	0.03	0.17	0.11	0.03
3-Split	0.16	0.95	0.28	0.15	0.05	0.22	0.12	0.05
9-Split	0.15	0.85	0.28	0.13	0.03	0.19	0.12	0.05

The usual foliar sampling period for diagnostic purposes in New Zealand is late February to March (Will 1985). The foliar nutrient concentrations at Bottle Lake in March indicate satisfactory levels for the growth of radiata pine (Table 4.10). Data for the Control and

Single treatments are presented because they had the highest and lowest concentrations respectively.

Table 4.10 Nutrient concentrations in 2.5-year-old radiata pine on March 27, 1984.\*

Treatment	P	K	Ca	Mg	S
	---- % oven dry weight ----				
Control	0.19	1.06	0.23	0.11	0.12
Single	0.15	0.89	0.22	0.11	0.10

\* : upper crown needles, approximately 6 months old.

There is an indication of lower concentrations on fertilized trees as with nitrogen (Figure 4.1). This could be a dilution effect due to the increase in foliage biomass (Chapter 3). Alternatively the fertilizer application may affect the availability of other nutrients in the soil (Chapter 7).

The following sections describe the seasonal changes in the foliar concentrations of phosphorus, potassium and magnesium. Needle nutrient contents are referred to but not presented. The foliar sulphur status and its interaction with nitrogen is discussed in Section 4.3.4.4.

4.3.4.1 Phosphorus There was an increase in foliar concentration on Controls in the spring (Figure 4.3). The Single treatment, however, continued to show a steady value which might have been a fertilizer effect. Both treatments show net retranslocation of phosphorus from 1982/83 needles in November. This coincides with the flush of new needles which initially had high concentrations (up to 0.30% P). These declined to a stable value by March (Table 4.10). The pattern for middle crown needles was similar but at a lower level. The seasonal patterns are similar to four other sites in New Zealand (Mead and Will 1976) and two in South Australia (Fife and Nambiar 1982). Unlike these studies, however, there was no apparent increase in autumn at Bottle Lake. The gradient between upper and middle crown needles was larger on Control trees in accordance with their higher concentrations (c.f. Section 4.3.2, Cromer *et al.* 1985). The N:P ratio in 1982/83 needles was constant at about 10 on Control trees. A rise was apparent on the Single treatment following fertilization. The N:P ratio increased steadily through the season in new needles, on Control trees, rising from 6.4 to 9.0 (Figure 4.3). The ratio in fertilized trees apparently fluctuated at a higher level. Fife and Nambiar (1984) showed similar fluctuations in the N:P ratios, which were attributed to different amounts of retranslocatable nitrogen and phosphorus. In this

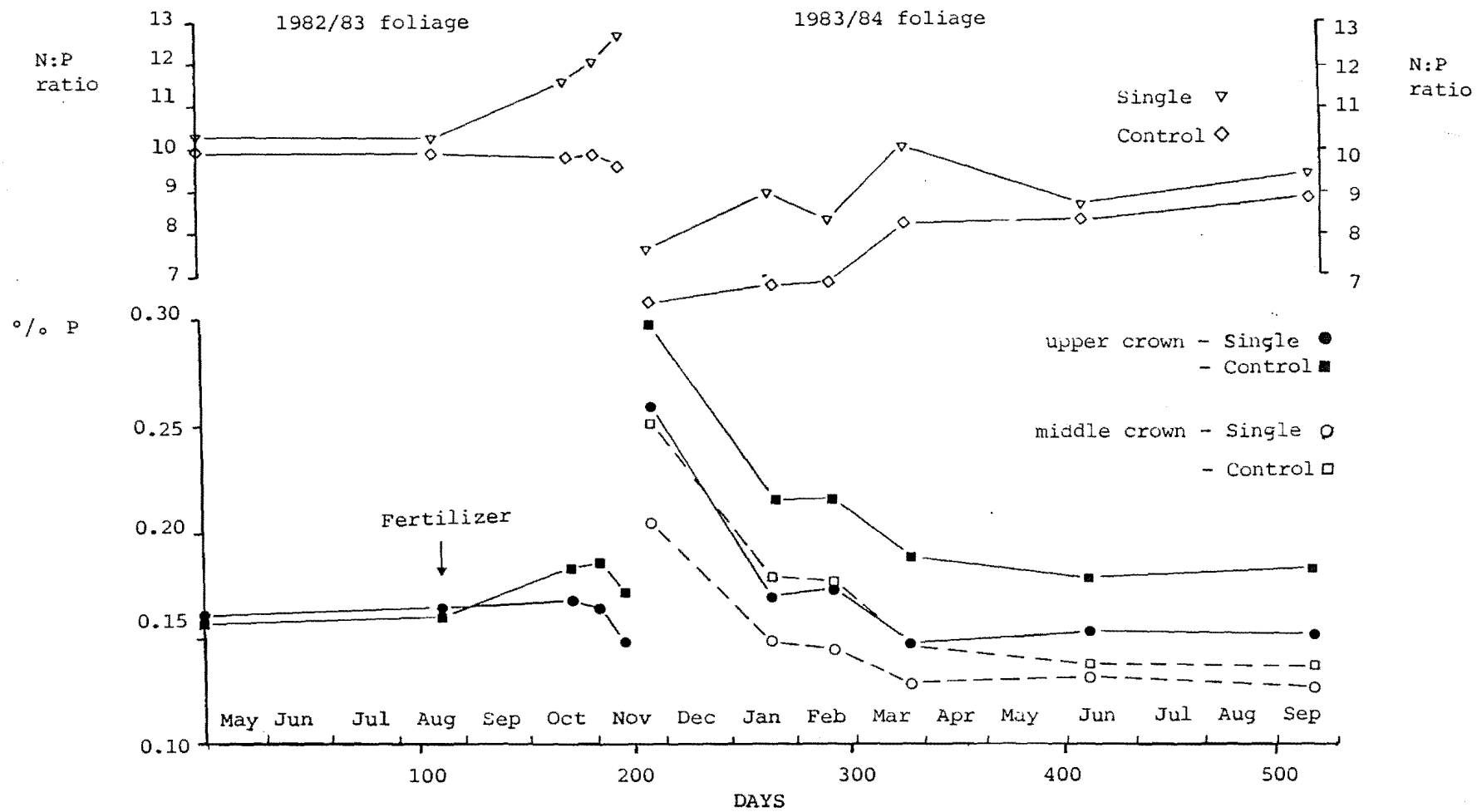


Figure 4.3 Foliar phosphorus status

study no net retranslocation of either element from these needles was apparent.

The optimum N:P ratio for the growth of radiata pine is reported to be 10 (Raupach *et al.* 1969). However, this will vary with site productivity and location (Lambert 1985).

4.3.4.2 Potassium Potassium concentrations in the new foliage declined throughout the year (Figure 4.4). Middle crown needles had lower concentrations. Potassium is more prone to foliar leaching than most nutrients which may explain some of the patterns (see Figure 2.1). The decrease from August to October in older foliage represents net loss of potassium from needles. This is unlikely to result from retranslocation to new tissues given the subsequent rise at a time when new needles are known to be forming. The decline from June to September 1984 also represents net loss of needle potassium. The steady decline in potassium contrasts with the results of Mead and Will (1976) and Fife and Nambiar (1982) who show a rapid decline followed by increases in late summer and autumn.

4.3.4.3 Magnesium There was a decline in magnesium concentration in the 1982/83 foliage (Figure 4.5). This represented net retranslocation of magnesium from these needles as the new foliage expanded. Limited yellowing of 1982/83 needle tips was noted on some trees during October and November which was considered to be withdrawal of magnesium. The decline in magnesium appeared more pronounced on the fertilized trees and may be due to a competitive effect between the uptake of  $\text{NH}_4^+$  and  $\text{Mg}^{++}$  (Mengel and Kirkby 1982, p.462).

The concentration of magnesium initially increased in young expanding needles. This contrasts with nitrogen, phosphorus and potassium, which declined, but is the same as calcium (data not presented). Accumulation was greatest in the middle crown needles. The differences between treatments were negligible (Figure 4.5). The lower values in September represent net loss from the needles which may be attributed to retranslocation to new tissues as in the previous spring. Yellowing of needle tips was again apparent on some trees at this time.

Of the four elements (N, P, K, Mg), magnesium was the only one to be retranslocated from the 1983/84 needles. This occurred in response to the demands for new foliage growth and resulted in marginal levels for magnesium. However, Will (pers. comm.) considered the observed needle chlorosis to be of minor significance. Photosynthesis might have been affected but the withdrawal of magnesium was from foliage which would be rapidly shaded by the new growth. It should also be noted that the 1982/83 foliage did not suffer a net drop in magnesium concentration from November, 1983 to October, 1984 (Table 4.11).

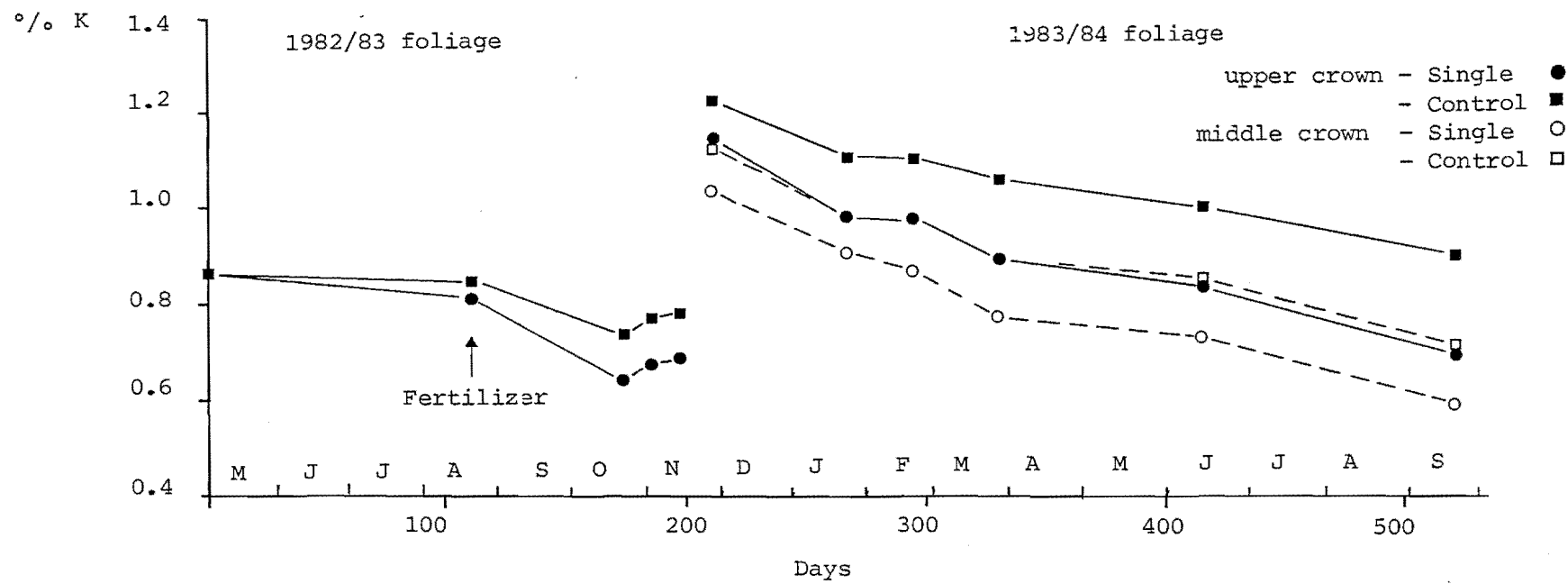


Figure 4.4 Foliar potassium concentration

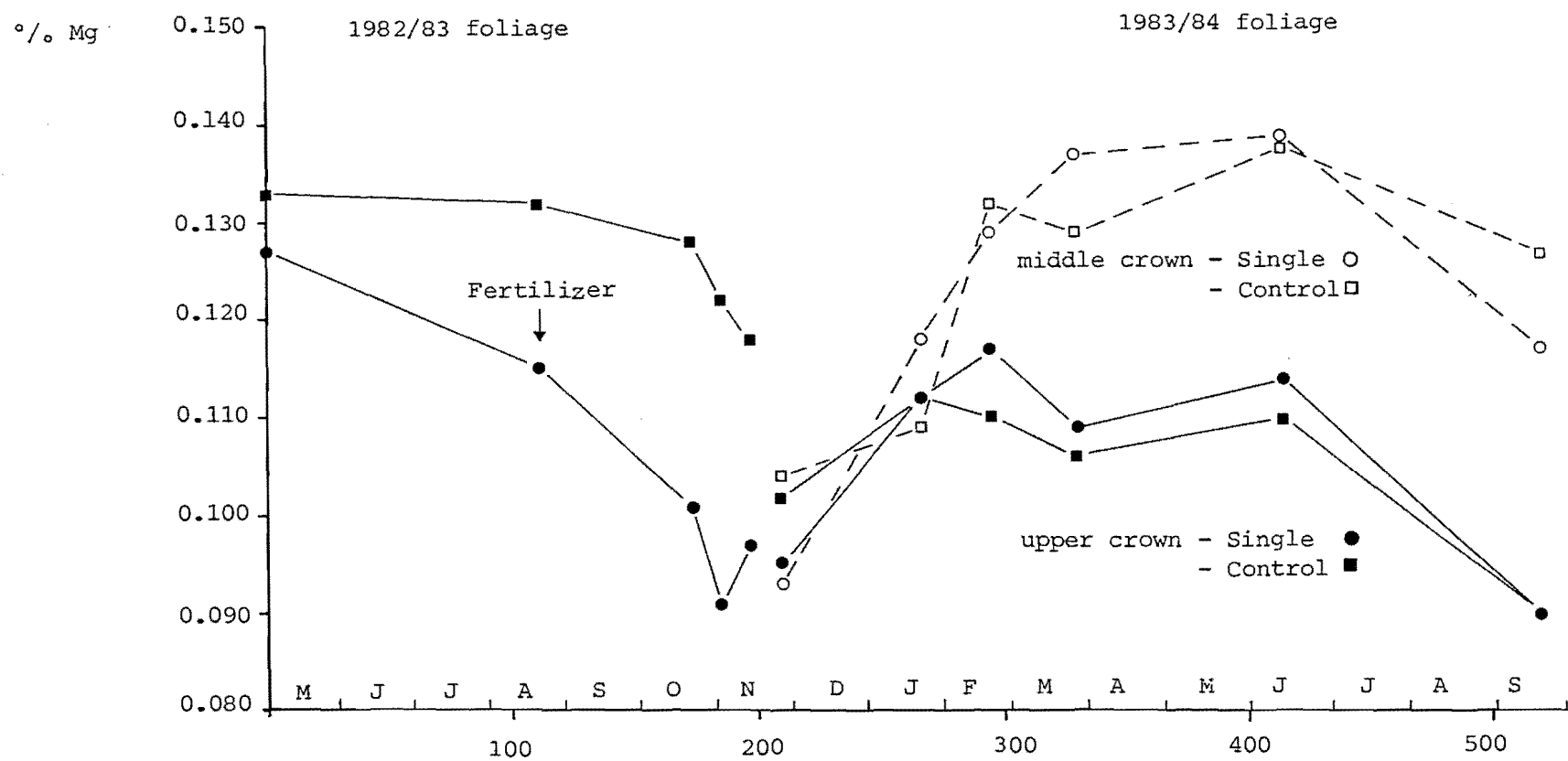


Figure 4.5 Foliar magnesium concentration



Table 4.11 Foliar magnesium concentrations.

Treatment	Nov. 1983	Oct. 1984
	----- % Mg -----	
Control	0.118	0.117
Single	0.097	0.117

4.3.4.4 Sulphur : Nitrogen Interactions Sulphur is an essential element for plant growth. The three sulphur containing amino acids, cystine, cysteine and methionine are essential for protein synthesis and make up 90% of the plant's organic sulphur (Turner 1979). The ratio of organic sulphur to organic nitrogen has been shown to be constant in radiata pine foliage (Kelly and Lambert 1972). This ratio is equivalent to the atomic ratio in foliar protein, which on a gram atom basis is 0.030, i.e.

$$\frac{\text{Organic S}}{\text{Organic N}} \times 0.437 = 0.030$$

If there is excess sulphur to balance the available nitrogen then sulphate ( $\text{SO}_4^{--}$ ) accumulates in the foliage (Humphreys *et al.* 1975). When nitrogen uptake commences, e.g. from fertilizer, then this pool of sulphate is used to form amino acids. The sulphate status of foliage has been proposed as a diagnostic tool in determining the effectiveness of applied nitrogen by Turner *et al.* (1977), who give critical levels for sulphate (Table 4.12).

Table 4.12 Critical foliar sulphate levels in winter.

Foliar sulphate (ppm)	Sulphur status
0 - 80	Deficiency to incipient deficiency
80 - 200	Marginal to adequate
200 - 400	Adequate to high
400+	High (possible nitrogen deficiency)

source: Turner *et al.* 1977.

Trees with a high foliar sulphate level would be expected to respond well to nitrogen fertilizer while those with little sulphate may show a nil or low response.

In this study the foliar sulphur status was initially investigated on the final biomass samples (Table 4.13).

Table 4.13 Sulphur and nitrogen status of 1983/84 foliage at the end of the experiment (Oct. 1984).

Treatment	Total N ----- % -----	Total S -----	S : N	SO <sub>4</sub> <sup>2-</sup> (ppm)
Control	1.604	0.141	0.039	310
Single	1.488	0.107	0.031	50
3-Split	1.454	0.121	0.036	212
9-Split	1.623	0.129	0.035	176

The sulphate levels were calculated from equations (1) and (2). This assumed that total nitrogen in the foliage is all in the organic form and that the ratio of organic sulphur to organic nitrogen is indeed 0.030.

$$\text{Organic S} = \frac{\text{Total N} \times 0.030}{0.437} \quad (1)$$

$$\text{so Inorganic S (SO}_4^{2-}) = \text{Total S} - \text{Organic S} \quad (2)$$

Analyses of variance showed that there were no significant differences between treatments for total nitrogen. The total sulphur and S:N ratios were significantly ( $p < 0.05$ ) lower on the Single treatment than on the Control and Split treatments. The initial sulphur status was the same across treatments (Table 4.9). It appears that there could be marginal sulphate levels on the Single treatment which may have been induced by the urea application.

The S:N interaction was investigated further using the seasonal foliage samples. The results are given in Appendix 9 and presented graphically for the Control and Single treatments (Figure 4.6).

The sulphur concentration rose in the 1982/83 foliage until November (Figure 4.6a), when a decline was apparent as with nitrogen (Figure 4.1). The pattern in the 1983/84 foliage was also similar to nitrogen. The sulphur content ( $\mu\text{g S/needle}$ ) (Figure 4.6b) also showed a

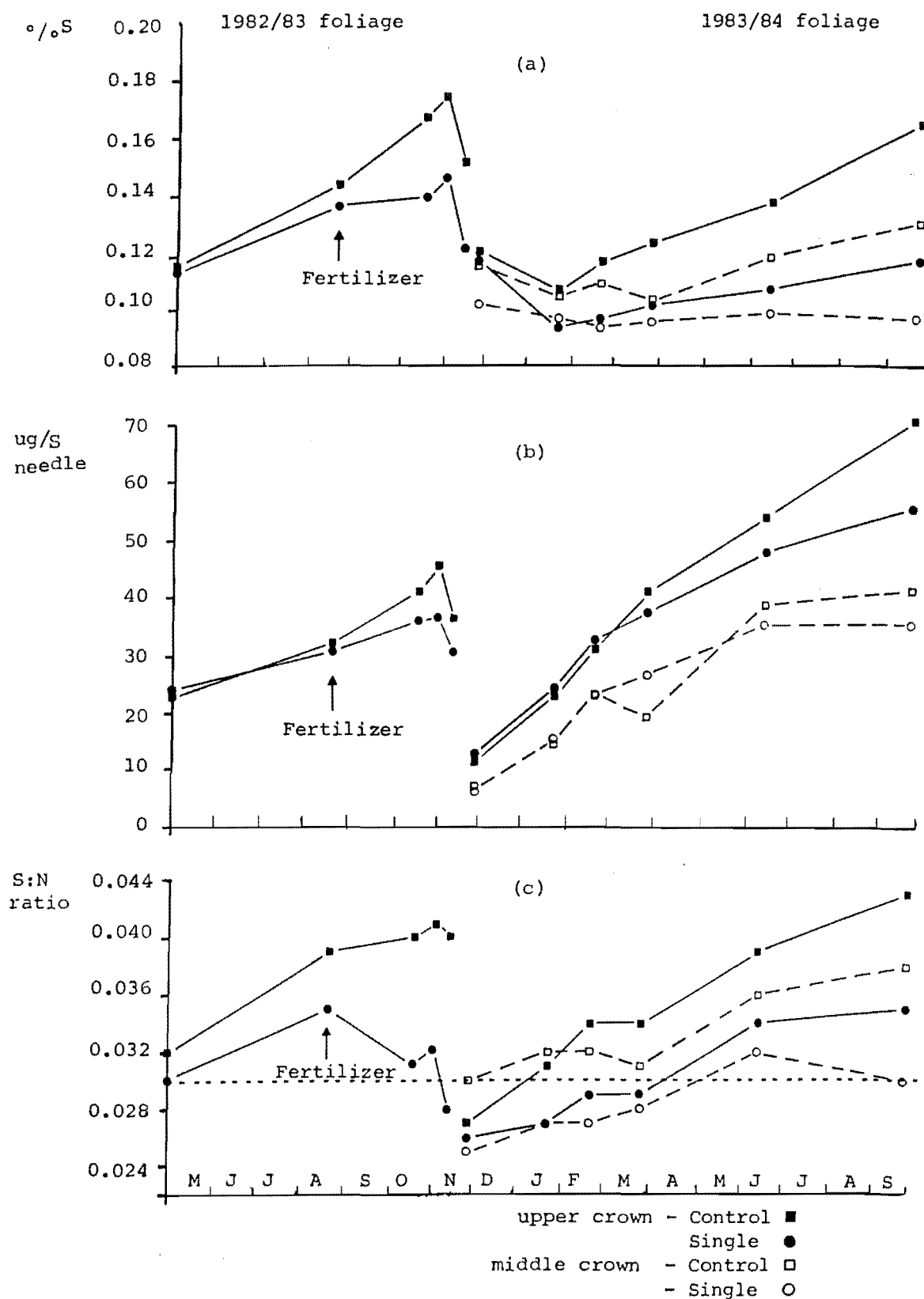


Figure 4.6 Foliar sulphur status (a) concentration, (b) needle content, (c) S:N ratio

similar pattern to nitrogen (Figure 4.2). The decline in 1982/83 foliage in November was net retranslocation, presumably to the newly expanding foliage. There was an indication of a lower sulphur accumulation in the needles after fertilizer was applied in August, 1983.

The S:N ratio is shown in Figure 4.6c with the organic S:N ratio of Kelly and Lambert (1972) as a reference. This ratio has not been confirmed in New Zealand radiata pine plantations, and it should be stressed that no organic sulphur determinations have been made in this study. Bearing this in mind, it is suggested that values  $> 0.030$  represent sulphate accumulation, i.e. sulphur is present in excess of the nitrogen available for amino acid synthesis. If the ratio is  $< 0.030$ , then sulphur is limiting and nitrogen is in "excess". Nitrogen has not been found in the inorganic form in radiata pine foliage. It is probable that the tree stores excess nitrogen as amino acids which do not contain sulphur, i.e. arginine, asparagine or aspartic acid (Turner *et al.* 1977).

In the 1982/83 foliage the ratio increased during the winter as sulphate accumulated (Figure 4.6c). This trend continued in the spring for Control trees. In contrast the Single treatment showed a decline after fertilizer was applied in August. Nitrogen was taken up and sulphate levels declined as amino acids were synthesized.

In the 1983/84 foliage the ratio increased throughout the year. There is an indication of inadequate sulphur levels on the Single treatment.

The actual sulphate levels were calculated according to equations (1) and (2) (see Appendix 9). Values for June of each year are given (Table 4.14) which may be compared with published critical levels in winter (Table 4.12). A possible sulphur deficiency was apparent on the Single treatment trees. The other treatments had adequate levels of sulphur.

Table 4.14 Estimated foliar sulphate levels in winter at Bottle Lake.

Treatment	June 27, 1983	June 20, 1984	
		upper	middle
	----- ppm $\text{SO}_4^{--}$ -----		
Control	154	320	189
Single	76	120	66
3-Split	237	215	-
9-Split	338	-	-

In conclusion, sulphate levels decreased after urea was applied, in agreement with Humphrey *et al.* (1975). The apparent zero sulphate levels on the Single treatment in the spring and summer of 1984 might have been expected to have some effect on the uptake and utilisation of nitrogen. However, there appears to have been no effect on nitrogen uptake (Figure 4.2). Also there was no detectable difference in the response to the Single application of nitrogen (Chapter 3).

Recently, Lambert (1986) has shown growth to be depressed following an application of 400 kg N/ha to 4-year-old radiata pine. This was attributed to induced sulphur deficiency. However, addition of sulphur in an adjacent plot yielded no growth response, although sulphate levels were increased. The S:N interaction requires further study, particularly, the question of whether a response to sulphur can be achieved in stands with either a natural or an induced deficiency of the element.

## CHAPTER 5

## MOVEMENT OF FERTILIZER N IN THE ECOSYSTEM

The movement of the applied fertilizer N into and within the tree was monitored by foliage sampling (c.f. Chapters 3 and 4). Some supplementary data were also collected to assess ammonia volatilization and leaching patterns. A few periodic soil samples were analysed for pH, nitrogen and fine roots.

## 5.1 INTRODUCTION

Following an application of urea to a soil and its subsequent hydrolysis (ureolysis) there are a number of pathways for the fertilizer N to enter the nitrogen cycle (c.f. Jansson 1971). Briefly these are:

- gaseous loss to the atmosphere,
- leaching to various depths,
- uptake by trees or other vegetation,
- immobilization by the soil microflora or on organic matter.

Added nitrogen is assimilated into the soil nitrogen pool where it will undergo the various mineralization, immobilization, nitrification and denitrification processes (Wollum and Davey 1975). However, this brief review focusses on the initial fate of applied urea.

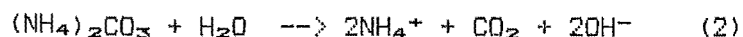
5.1.1. Hydrolysis

Once urea comes into contact with the soil it is rapidly converted to unstable ammonium carbonate by the enzyme urease.



Urease is sufficiently abundant in most soils to effect the hydrolysis of urea (Wollum and Davey 1975). The limiting factor in Equation 1 is usually the availability of moisture. When surface forest soils are susceptible to rapid drying, ureolysis may be retarded (Foster *et al.* 1980). In a New Zealand study using pumice soil from Kaingaroa Forest, rapid ureolysis was indicated by the minimal leaching of undissociated urea (Ballard 1979).

The ammonium carbonate in Equation 1 is further hydrolysed to ammonium and carbon dioxide.



The importance of this reaction is the production of hydroxyl ions, resulting in an alkaline environment in the vicinity of hydrolyzing urea. A rise of several pH units may be observed (Black et al. 1985). The possibility of volatilization (loss of ammonia gas) becomes important as the ammonium ion and molecular ammonia exist in a pH dependant equilibrium. Most forest soils have a pH <6.0, when only 0.1% of ammonia occurs in the molecular form. At pH 9 this has increased to 50%.

### 5.1.2. Volatilization

Numerous studies have estimated the loss of fertilizer nitrogen by volatilization and factors controlling it (see Terman 1979, Otchere-Boateng 1979). Losses of <5% (Overrein 1968) to >30% (Carrier and Bernier 1971) have been reported for forest ecosystems. Factors such as high application rate, warm temperatures, high wind velocity and low soil moisture content will tend to promote volatilization. Soils with relatively high pH and low C.E.C. will also be susceptible to ammonia loss. This loss usually occurs within a few days of application assuming ureolysis is rapid. Although some of the losses reported in the literature are disturbing, they should only be treated as "apparent losses." Plants have the ability to both release and absorb ammonia from the atmosphere (Mengel and Kirkby 1982, p.303). Pang (1984) has demonstrated the ability of douglas fir foliage to absorb gaseous ammonia. Clearly volatilization from the soil surface does not necessarily mean a loss of fertilizer N from the ecosystem.

Progress in assessing the importance of volatilization has been hindered by the methodology available for quantifying evolved ammonia. Marshall and DeBell (1980) reported that the large range of losses was probably due in part to the variety of methods employed. They tested four main methods:

- (i) closed static systems,
- (ii) semi-open systems,
- (iii) closed dynamic systems,
- (iv) N-15 balance.

The first three adopt a collection system for ammonia gas, usually an acid sorber. The first type relies on diffusion of ammonia from the soil to the sorber (e.g. Boomsma and Pritchett 1979). The second allows contact with the atmosphere (Nömmik 1973b, Carrier and Bernier 1971). The third simulates natural conditions by drawing air across the soil surface for collection (Watkins et al. 1972, Black et al. 1985). The N-15 balance method (Nömmik 1973a) relies on accounting for all other losses and sinks for nitrogen. The unaccounted for portion can only strictly be called loss of nitrogen gases, the species not

being identified. A further method of interest is the micrometeorological technique (Denmead *et al.* 1977). A mast with ammonia traps attached at various heights is set up downwind of a fertilizer field. Fluxes of ammonia across an assumed vertical plane are calculated from wind speed and ammonia concentration. The possibility of transferring this technology from agricultural to forest systems is however questionable.

Of the three sorber methods tested by Marshall and DeBell (1980), the closed static system gave the lowest estimate of ammonia loss, the closed dynamic system the highest. They concluded that the latter gave the most representative estimate of ammonia loss as this agreed with the N-15 balance method corrected for loss of other nitrogen gases.

Volatilization has not been measured in New Zealand forests but it is believed that conditions do not favour such losses (Will *et al.* 1980).

### 5.1.3. Leaching

The amount of nutrients leached from the rooting zone depends on the amount of water passing through the root zone, the mobility of the nutrient in the soil and the amount of uptake by roots and soil organisms (Sands 1984).

Undissociated urea is extremely mobile and subject to leaching; however, hydrolysis of urea applied to forest soils is usually very rapid (Cole *et al.* 1975). The ammonium ion produced is usually not susceptible to leaching as it is absorbed by clay and organic fractions in the soil. However, after urea application to sandy soils low in exchange sites appreciable quantities of the ion may be leached.

If nitrification occurs the added fertiliser may ultimately be leached as the anion  $\text{NO}_3^-$ . This may be critical for other nutrient cations in the soil,  $\text{K}^+$   $\text{Ca}^{++}$   $\text{Na}^+$  for example, as leaching is a coupling process with a cation and anion being transported together (Khanna and Ulrich 1984). High concentrations of  $\text{HCO}_3^-$  may also facilitate cation leaching. This anion is a product of the hydrolysis of urea (Cole *et al.* 1975).

In a laboratory study using cores of Kaingaroa pumice soil, Ballard (1979) found very little urea in leachates at 10 cm depth. Initially leaching was slow until nitrification occurred when large amounts of nitrate were leached. In conjunction with this the cations  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and  $\text{K}^+$  were also leached. Cole *et al.* (1975) using tension plate lysimeters in the field found the only leaching of urea was in the non-ionic form. However, most applied nitrogen remained within the top 15 cm of soil.

Measurements of leaching are rarely undertaken in field nitrogen balance studies following fertilization. This is no doubt due in large part to the methodological difficulties in collecting representative leachate samples, particularly if a quantitative estimate is required



(see Barton (1981) for a review). In some nitrogen balance studies the unaccounted for fertilizer has been attributed to leaching. Heilman *et al.* (1982b) were unable to account for  $32 \pm 6\%$  of 224 kg N/ha in a 5-7-year-old douglas fir stand. Some of this was considered to have been leached. In a 120-year-old scots pine stand Nõmmik and Möller (1981) accounted for 78% of 150 kg N/ha applied as urea. When ammonium nitrate was the nitrogen source much greater leaching losses were reported. In a study with ammonium sulphate Mead and Pritchett (1975b) were only able to account for 50% of the applied nitrogen. Leaching and gaseous losses were reasons invoked for this low recovery.

A large field lysimeter (Will 1977) was used to study the fate of 200 kg N/ha applied as a urea solution to a 13-year-old radiata pine stand (Worsnop and Will 1980). No fertilizer nitrogen could be detected at 2.7 m depth, but only 44% could be recovered within the top 30 cm of soil after 147 weeks. Although tree uptake was considered to be a major sink it would be surprising if this accounted for 56% of the applied nitrogen.

#### 5.1.4. Plant uptake

After ureolysis a high concentration of ammonium ion will temporarily be in solution. Plant uptake will be determined by the ability of ions to move to the roots and the ability of roots to absorb nutrients arriving at their surface (Bowen 1984). Ammonium moves by diffusion, the rate declining as soil dries. It is considered a poorly mobile ion, so uptake may largely depend on rooting density. This is reported to be low for young radiata pine on sand from planting to four years of age (Nambiar 1983). Root activity should be an important factor in the efficient use of fertilizers as uptake is an active process for most ions. Root initiation and elongation in radiata pine are greatly retarded below a critical root temperature between 11 and 14°C (Nambiar *et al.* 1979).

Ideal conditions for the application of nitrogen fertilizers are periods of high root activity, moderate temperatures, moist litter and soil conditions and a high probability of moderate rainfall within a few days of application (Ballard 1984). Because urea is prone to loss by volatilization (section 5.1.2.), applications are often based on climatic conditions rather than root activity. Heilman *et al.* (1979) recommend applying urea to douglas fir in late autumn or winter, primarily to ensure rapid incorporation into the upper soil layers and to prevent volatilization.

Because of the variability in foliar nitrogen concentrations between trees (Mead 1984) and the often small and transitory increases in foliar nitrogen following fertilization (e.g. Nambiar and Bowen 1986), the use of N-15 to monitor fertilizer uptake is particularly useful. Most studies utilising this isotope of nitrogen have sampled one or more ages of foliage at various positions within the crown to follow nitrogen uptake. Results have either been expressed as

atom % N-15 excess (Nõmmik 1966, Björkman *et al.* 1967), proportion of nitrogen derived from the fertilizer, Ndff (Mead and Pritchett 1975a, Worsnop and Will 1980, Melin *et al.* 1983), or the product of Ndff and total nitrogen concentration (Heilman *et al.* 1982a).

Fertilizer is quickly detected in the foliage: within 3 weeks (Heilman *et al.* 1982a), within 2 weeks (Worsnop and Will 1980) and after 1 week (Mead and Pritchett 1975a). Uptake, as indicated by the above parameters, is usually rapid for the first few months before a plateau is reached (c.f. Figure 3, Mead and Pritchett 1975a, Figure 1, Melin *et al.* 1983). The proportion of nitrogen derived from the fertilizer will usually decrease with time as fertilizer uptake ceases and dilution by native soil nitrogen occurs. This is particularly pronounced in young trees with rapidly expanding crowns (Nambiar and Bowen 1986).

#### 5.1.5 Immobilization of nitrogen

Immobilization and the opposite process, mineralization occur simultaneously in forest soils, although the nitrogen equilibrium is generally in the direction of immobilization (Wollum and Davey 1975). Following urea application a large proportion of fertilizer nitrogen is retained within the litter layer and upper soil horizons (Overrein 1972, Worsnop and Will 1980, Nõmmik and Möller 1981). This immobilization of nitrogen may be caused by either abiotic or biotic factors (Wollum and Davey 1975). Microbial immobilization is commonly cited as the main process (Nõmmik and Popovic 1971); however, in some studies chemical fixation of ammonia predominates (Foster *et al.* 1985a).

This tendency for urea N to become immobilized has some benefits in terms of retention of fertilizer nitrogen within the ecosystem. However, unless the quantity of nitrogen applied is large in relation to the original nitrogen pool, or mineralization is increased, this advantage is unlikely to provide appreciable additional nitrogen after initial fertilizer uptake ceases (Mead and Gadgil 1978). Miller (1981) describes this concept as fertilizers being generally of benefit to the tree, not to the site. Other sources of nitrogen such as ammonium nitrate have a lower retention within the soil and often give better growth responses than urea (Nõmmik and Möller 1981). High immobilization of urea nitrogen is one reason for its replacement by ammonium nitrate in Scandinavian forest fertilization programmes. Elsewhere these two nitrogen sources have given similar responses (see Ballard (1984) for a review).

## 5.2. METHODS

### 5.2.1. Volatilization

A simple design of "closed static sorber" (Marshall and DeBell 1980) similar to that of Boomsma and Fritchett (1979) was used. Boric acid treated filter papers were placed in 250 ml beakers or petri dishes and inverted on the plots. Wires were used to prevent papers falling to the soil. The beakers contained 70 mm diameter filter papers treated with 0.8 ml of 3% boric acid and were 80 mm above ground level. The petri dishes contained 90 mm diameter papers treated with 1.0 ml of 3% boric acid and were 10 mm above ground level.

Four sorbers, two of each type were placed randomly on the plots after the summer fertilizer application. They were replaced at 3, 6, 15, 34, 58 and 113 days after the summer application with fresh sorbers located on a different spot. Filter papers were returned to the laboratory and frozen until analysis could proceed. Four sorbers were also placed on plots after the autumn and spring fertilizer applications. They were only analysed for the first sampling at day 3.

The filter papers were analysed according to the method of Keeney and Nelson (1982) for inorganic nitrogen. They were torn up, added to a Kjeldahl flask along with magnesium oxide and steam distilled. The liberated ammonia was collected in boric acid and titrated with 0.005 N  $\text{H}_2\text{SO}_4$  to determine  $\mu\text{g N}$  per filter paper. This rarely exceeded 50  $\mu\text{g N}$ . Accordingly care was taken to ensure that appropriate blanks were run and that the still was thoroughly clean.

### 5.2.2. Leaching

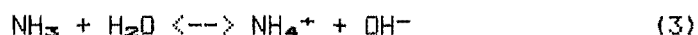
Downward movement of fertilizer was monitored by collecting leachates from fertilized and control plots and comparing them for ammonium content.

Small porous cup lysimeters were constructed to extract soil water (Talsma *et al.* 1979). Three lengths of lysimeters were made so as to collect samples from 20, 40, and 80 cm depth. A total of 42 lysimeters were installed (7 treatments  $\times$  3 depths  $\times$  2 replicates). This was easily accomplished by making a hole with a steel rod, almost to the correct depth, inserting the lysimeter, then pushing it in the last few centimetres to achieve a firm contact between the sand and the porous cup.

Water was extracted by connecting the lysimeter to an evacuated container. Three methods of collection were tried: a testube in an evacuated milk bottle, an evacuated boiling tube, and commercial vacutainers. Samples were collected by all methods but the boiling tube was preferred because of the subsequent analytical method. The boiling tubes were evacuated using a small hand pump, and a bicycle tyre valve retained the 0.3 bar vacuum.

Samples could only be collected when the sand was sufficiently

moist. Consequently collection times and lengths were erratic depending on rainfall. Samples were returned to the laboratory and if not analysed immediately they were stored at 4°C. Analyses were performed with an HNU ammonia gas sensing electrode connected to an Orion digital mv/pH meter. Samples were analysed relative to a calibration curve prepared from standard ammonium chloride solutions between  $10^{-2}$  and  $10^{-7}$  M  $\text{NH}_4^+$ . The electrode detects ammonia gas rather than the ammonium ion so the equilibrium in Equation 3 had to be shifted to the left.



This was achieved by adding 10 N sodium hydroxide just prior to the measurement. The ratio of sample to sodium hydroxide used was 100:1 by volume. The electrode fitted neatly into the boiling tubes used for collection. The analyses were performed in a controlled environment because the method utilises the Nernst equation which is temperature dependent. It is apparent that the method releases gaseous ammonia so no further nitrogen analyses were possible on the samples.

The volume of leachate collected was often barely adequate for ammonia analysis to proceed. However, on a few samples nitrate was analysed as well using an Orion specific nitrate electrode. No analyses for possible undissociated urea were made.

### 5.2.3. Foliar N-15 Analysis

Foliage sampling procedures are described in Chapter 3, and initial chemical analysis in Chapter 4. After titration for total nitrogen the sample was acidulated with 2 ml 0.08 N  $\text{H}_2\text{SO}_4$  (Hauck 1982), and then evaporated to dryness. Great care was taken during the preparation of samples (c.f. Hauck 1982, Reeder *et al.* 1980, Reeder 1984, Buresh *et al.* 1982). Ethanol was distilled between samples, and glassware was rinsed in dilute hydrofluoric acid.

After drying the samples were transferred to sampling vials for the mass spectrometer. Initially the G.P. Mass Spectrometer at the Institute of Nuclear Sciences, Lower Hutt, was used. This was built in the Dominion Physical Laboratory in the early 1950s (Hulston and Shilton 1958).

The dried sample of ammonium sulphate (>1 mg N) with attendant boric acid contamination was converted to dinitrogen gas using lithium hypobromite (Ross and Martin 1970). Precautions discussed by Porter and O'Deen (1977) and Hauck (1982) were also observed. Samples were analysed relative to a cylinder of dinitrogen gas. Standards prepared from the original fertilizer and naturally enriched urea were also analysed. Unfortunately, increasing problems and a final breakdown forced the abandonment of this machine.

Samples were subsequently analysed by Professor W.B. Silvester at the University of Waikato. The initial sample preparation was changed

to provide samples with 200 ug N and a lower boric acid content. Standards prepared for the first mass spectrometer were run on the Waikato machine to check for comparability. The agreement was satisfactory.

The results have been presented as the percentage of nitrogen derived from the fertilizer (% Ndff):

$$\% \text{ Ndff} = \frac{c - b}{a - b} \times 100$$

where a: atom % N-15 in the original fertilizer solution.

b: atom % N-15 in unfertilized trees (natural abundance).

c: atom % N-15 in fertilized trees.

#### 5.2.4. Soil Analysis

Soil samples were collected from plots beginning just prior to the Single treatment application in August. A Hoffer soil tube was used to take three cores per plot to a depth of 30 cm. These were split into 0-10 and 10-30 cm and bulked by plot. Samples were collected on the following dates:

18th August	1983
18th October	1983
12th December	1983
7th February	1984
20th June	1984

Samples were returned to the laboratory and air dried.

5.2.4.1 pH pH was determined in a slurry of 10 g soil in 25 ml distilled water (Nicholson 1984). Samples were left covered overnight at 20°C prior to analysis with a compact glass electrode.

5.2.4.2 Nitrogen Analysis for total nitrogen and N-15 were performed for the Single treatment plots only. Trends in N-15 for this treatment from the time of application onwards should give an indication of fertilizer fate in the soil and be comparable with other studies adopting a conventional single application. Roots were removed from samples using a 0.5 mm sieve. Samples were then finely ground in a Rocklabs ring grinder.

Total nitrogen was determined after digestion by the salicylic acid-thiosulphate modification of the Kjeldahl method (Bremner and Mulvaney 1982). 0.5 g samples were placed in digestion tubes and 4 ml salicylic/sulphuric acid mix added. These were left overnight before adding 0.5 g sodium thiosulphate. After cooling, one sodium sulphate/selenium tablet was added. Digestion commenced as described

in Chapter 4, until "clearing". The afterboil period was extended to four hours. After cooling and diluting, the whole sample was transferred to a Kjeldahl flask and distilled. Total nitrogen was determined as for foliage (Chapter 4), except 0.005 N standardised acid was used for titration. Preparation for N-15 analyses were as for foliage (Section 5.2.3).

5.2.4.3 Fine Roots For the 3-Split and 9-Split treatments, roots were extracted from the 10-30 cm soil sample and analysed for nitrogen. There were insufficient roots in the 0-10 cm sample for analysis.

The soil sample was spread on a 0.5 mm sieve to remove the bulk of sand. Material remaining was spread on a sheet of paper and sorted for roots and mycorrhizal tips. These were picked out using forceps and bulked by treatment for each of the five sampling dates. The roots in these small cores were usually < 1 mm in diameter. The few woody roots > 2 mm were discarded as they would probably have induced a bias in the results.

The roots were finely ground and 0.2 g taken for total nitrogen and N-15 analysis using the methods described for foliage (sections 4.2.3 and 5.2.3). A subsample was ashed to correct for sand contamination, so %N is reported on an ash free basis.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Volatilization

Losses of ammonia were negligible, the cumulative loss for 113 days after the summer application being a maximum of 1.3% of the nitrogen applied. This was to be expected considering the urea was applied as a solution. Volk (1970) reported a loss of ammonia totalling 0.1% of urea solutions applied to soils under slash pine after 7 days. This agrees with the figures in this study. Even accounting for the possible underestimates using a "closed static sorber" (Marshall and DeBell 1980) the losses would have been no more than a few per cent of that applied.

The pattern of ammonia loss is shown in Figure 5.1. The peak loss within the first few days is in agreement with other studies (Boomsma and Pritchett 1979, Overrein 1968). In view of the negligible losses any differences between treatments should only be viewed as indicative of the processes involved.

The loss of ammonia from the seasonal treatments within 3 days was 0.16, 0.03 and 0.07% for the Autumn, Spring and Summer treatments respectively. The higher loss in autumn coincided with a lack of rainfall following the application (Table 2.4, Chapter 2). The importance of rainfall in reducing volatilization is widely acknowledged (Marshall and DeBell 1980, Ballard 1984, Will 1985).

In August and December 30 g N were applied to the appropriate

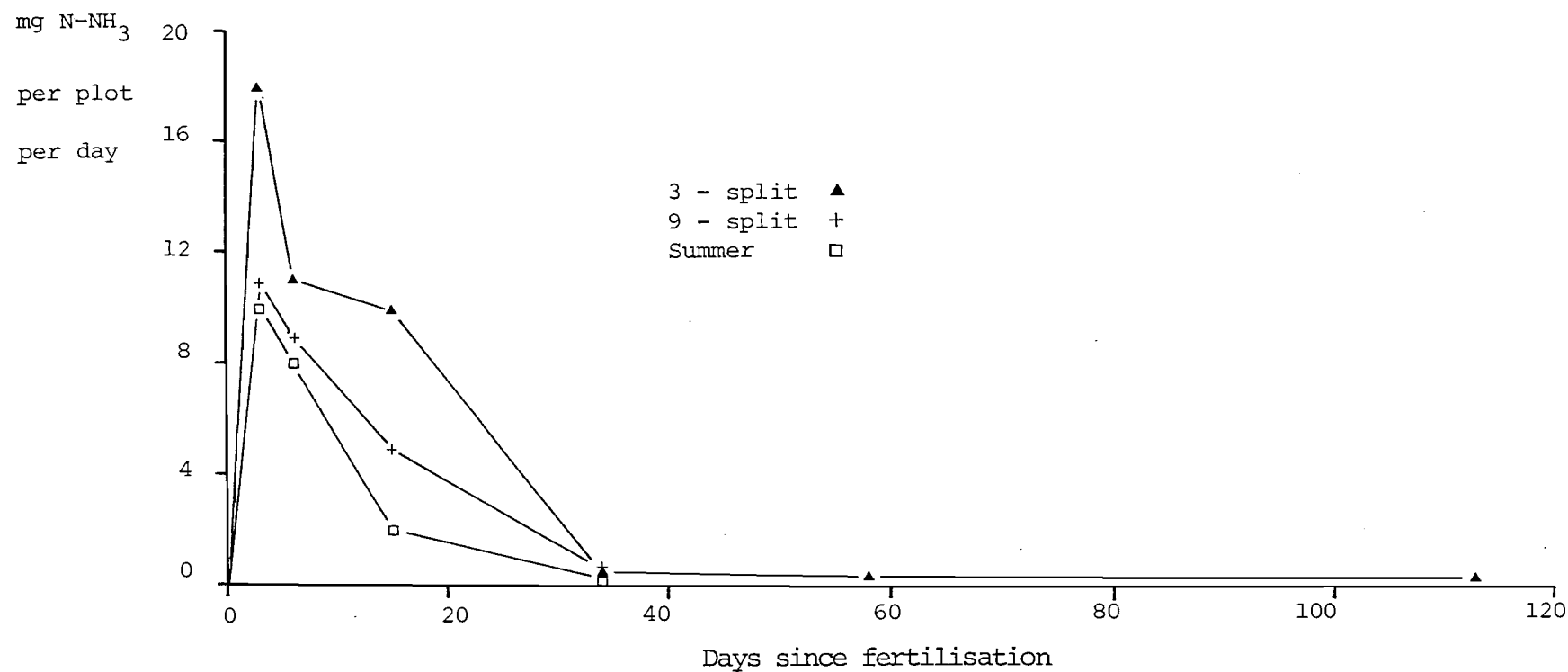


Figure 5.1 Average daily volatilization rate plotted against day of sampling, following summer fertilizer application on 13 December 1983.

seasonal treatment plots and the 3-Split treatment. More ammonia was volatilized from the latter suggesting enhanced loss from plots which had previously received fertilizer. Comparing the cumulative loss for 113 days following the last fertilizer application in December confirms this (Table 5.1).

Table 5.1 Cumulative loss of ammonia for 113 days following the last fertilizer application on December 13, 1983.

Treatment	g N applied	Number of previous applications	Loss - % of applied
Summer	30	0	0.3
3-Split	30	2	0.5
9-Split	10	8	1.3

Urea applications alter the chemical and biological status of the upper soil layers. Elevated levels of pH, urease activity and ammonium could lead to greater losses from subsequent applications. Black *et al.* (1984) found a greater loss of ammonia from urea applied to sheep urine patches than to unaffected pasture. They considered surface soil pH and ammonium levels to be critical factors in this increased loss. Marshall and DeBell (1980) cite unpublished data showing that the previous history of soil fertilization affects gaseous loss.

In this study the greater loss from split applications is insignificant in terms of the overall nitrogen balance sheet. However, if prilled urea, rather than the solutions used here, were applied, then the possibility of significantly increased loss of ammonia should be considered.

### 5.3.2 Leaching

5.3.2.1 Ammonium The concentration of ammonium expressed as ppm is shown in Figures 5.2 and 5.3 for the main and seasonal treatments. The Control data are included on both. Note: the ordinate axis is logarithmic.

For all depths the Control plots had ammonium concentrations at or below 0.1 ppm. This agrees with values under a 30-year-old radiata pine stand in Kaingaroa Forest (Dyck *et al.* 1981).

Levels above Controls are assumed to be ammonium derived from the fertilizer. This may not be valid if fertilizer promotes mineralization, a priming effect (Popovic and Nõmmik 1972, Heilman *et al.* 1982a). However, as an indication of fertilizer moving through the profile this assumption is justified. The pattern of leaching is briefly discussed for each treatment.



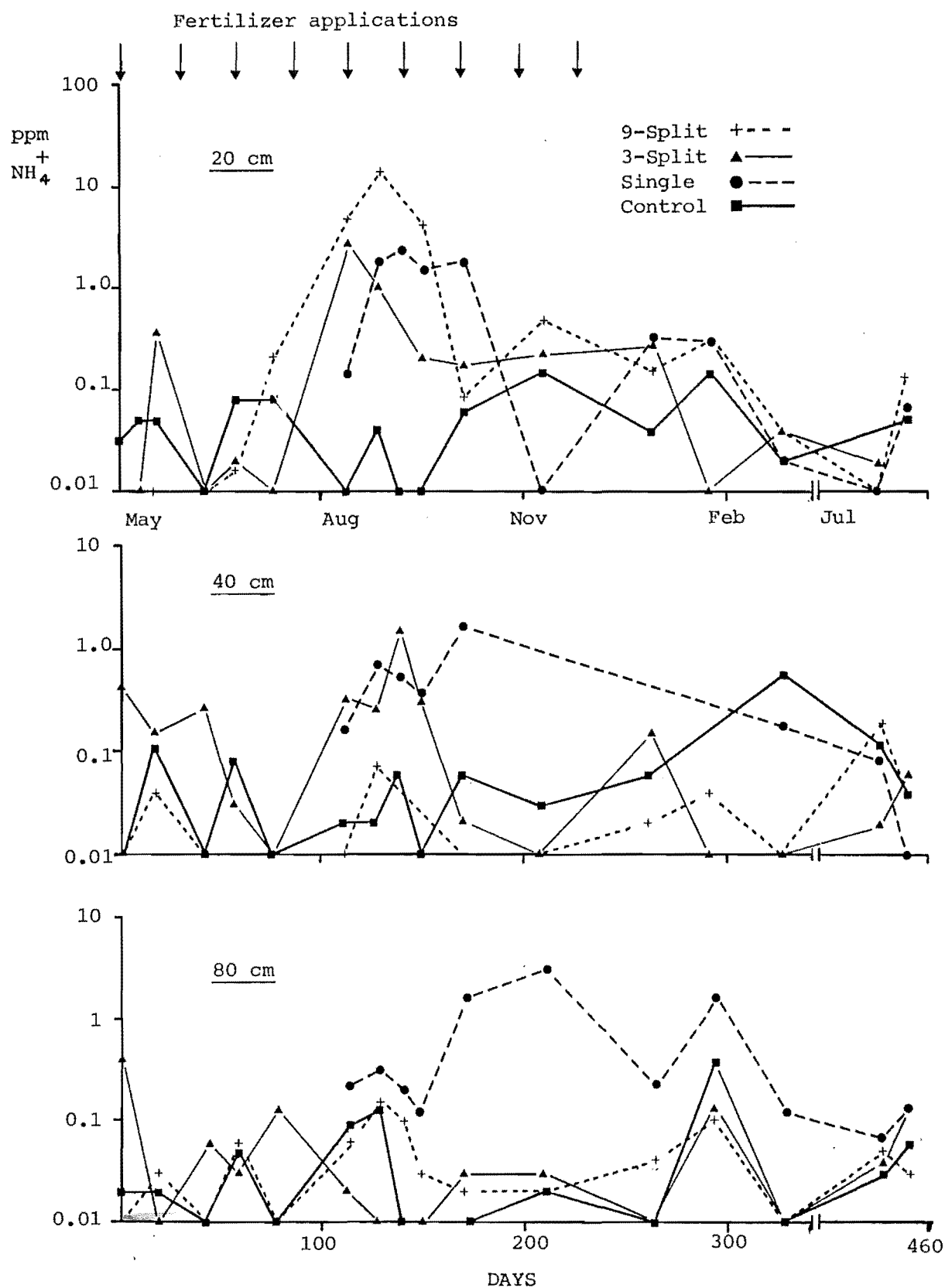


Figure 5.2 Ammonium concentration in main treatment - leachate samples at 20, 40, and 80 cm depths

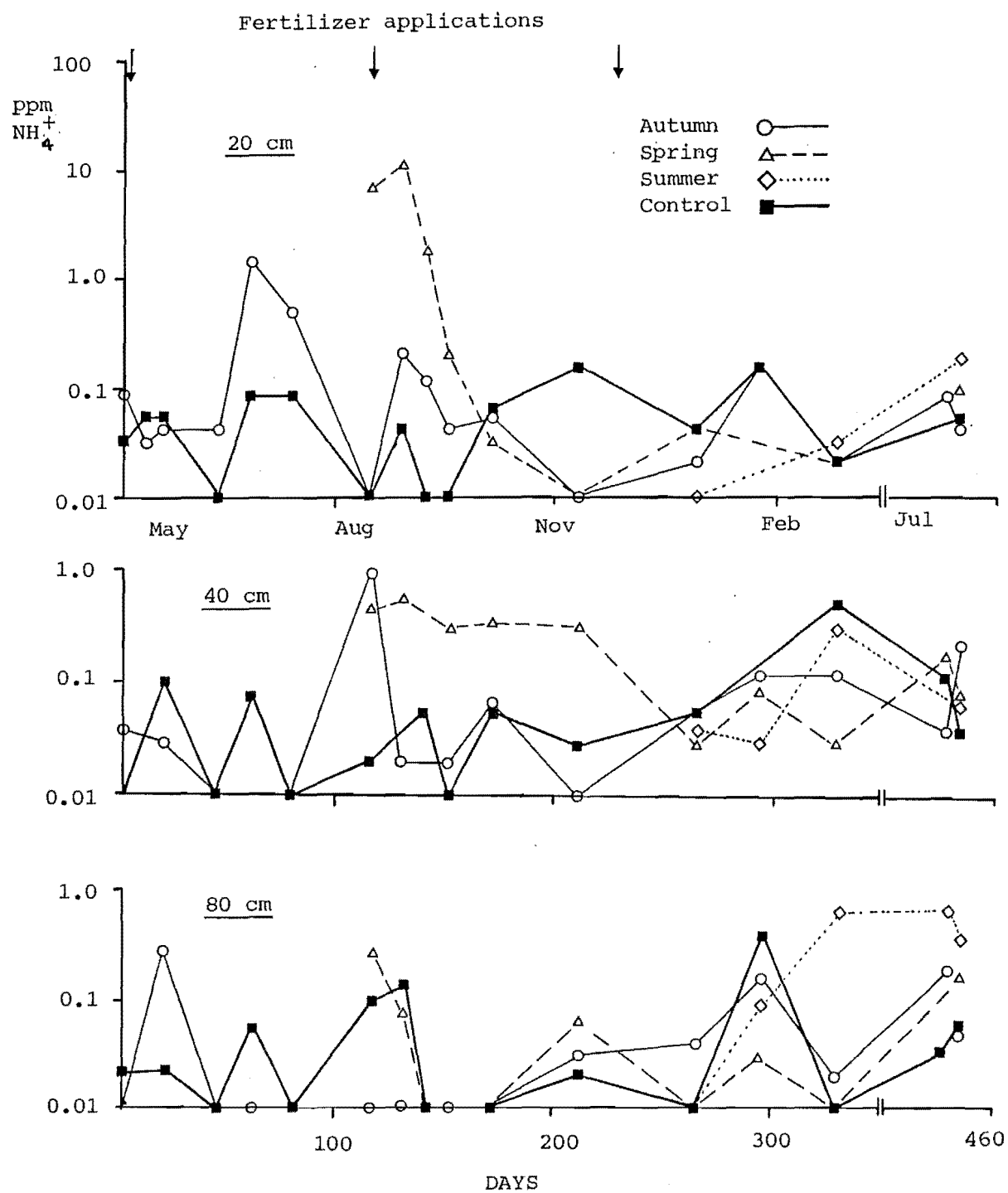


Figure 5.3 Ammonium concentration in seasonal treatment leachate samples at 20, 40, and 80 cm depths

Single This application of 90 g N was applied on August 22, a day on which 2.5 mm rain fell. A further 15 mm fell in the next two days (Table 2.4). A collection made during this time detected fertilizer at each depth (Figure 5.2). Concentrations generally remained above Controls until about March. The quantity of ammonium leached cannot be calculated and comparisons with other studies are difficult given peculiarities of site and sampling.

3-Split Three small peaks were apparent at 20 cm corresponding with fertilizer applications. The same pattern, but up to 30 days later, was observed at 40 cm. There was no appreciable leaching of fertilizer to 80 cm depth.

9-Split Fertilizer was not detected at 20 cm until after the third application on June 27, when 32 mm rain fell in the following week. Thereafter elevated ammonium levels were detected until March. There was no evidence for appreciable leaching to either 40 or 80 cm depth for this treatment.

Autumn A peak was evident some 60 days after fertilizer application at 20 cm. Within 100 days Control levels were reached. Two peaks at 110 and 18 days indicate that fertilizer may have leached to 40 and 80 cm.

Spring This application of 30 g N coincided with the Single application of 90 g N. A similar pattern was seen except Control levels were reached sooner at 20 and 80 cm depth.

Summer On the day of application 3.5 mm rain fell and 47 mm within the next 3 days. Unfortunately no collection was made until day 36. For the remainder of the experiment elevated levels were apparent at 80 cm.

There was evidence for two periods of leaching following urea application:

- (i) immediately afterwards with a significant rain event, e.g. Single and Spring treatments when fertilizer was detected within 2 days at 80 cm.
- (ii) within a period of about 200 days with a tendency for peaks to occur in the order of 20, 40, 80 cm.

There was an indication of greater leaching in the Single as opposed to Split application treatments. For the Single treatment Control levels were reached about 210 days after application. Worsnop and Will (1980) report a return to pretreatment ammonium levels, at 30 cm depth 270 days after applying 200 kg N/ha as urea to a 13-year-old radiata pine stand. Heilmen *et al.* (1982a) applied urea at 224 kg N/ha to young douglas fir. After an initial peak, the ammonium content of the soil to 38 cm depth declined rapidly and control levels

approached after 170 days.

**5.3.2.2 Nitrate** The nitrate ion was only analysed on a few plots from February to July, 1984 (data in Appendix 10). Concentrations of nitrate were often at least an order of magnitude higher than the ammonium ion. Concentrations in the Control varied from 0.5-2.5 ppm  $\text{NO}_3^-$ . These are considerably higher than a control plot under radiata pine in Kaingoroa Forest (Dyck *et al.* 1983). Their levels averaged 0.006 ppm for 2 years. The Bottle Lake site apparently has a high nitrifying capacity. Nitrogen is reputedly conserved in undisturbed systems so the experimental conditions may be responsible for these high levels. Vegetation was controlled by herbicide, thus removing a possible sink for nitrate. It should also be noted that the previous pine crop was windblown in 1978 and not replanted until 1981.

High levels of nitrate up to 24 ppm were detected on some fertilized plots. There were no apparent trends across treatments. Concentrations were generally higher at the lower depths and a decline from February to July was apparent on some plots. These high levels were confirmed by repeating the analysis using an autoanalyzer.

There was apparently a population of nitrifying bacteria on this site which utilised some of the ammonium pool. The movement of nitrate to depth is a loss of nitrogen from the major rooting zone. Cations may also have been lost in association with the nitrate ion. The implications of leaching are discussed in Chapter 7. The period of nitrification following fertilization cannot be determined from these results. However, high nitrate levels as ammonium was declining may suggest that nitrification was delayed. This was the case in laboratory studies (Ballard 1979) and under a 23-year-old radiata pine stand following urea applications (Adams and Attiwill 1983).

### 5.3.3 Foliar N-15

**5.3.3.1 Percentage of Nitrogen Derived from the Fertilizer (% Ndff).** The uptake patterns for the main and seasonal treatments are shown in Figures 5.4a and 5.4b. Analyses of variance were initially performed using data at 14, 55 and 110 days since the first applications (Tables 5.2 and 5.3). These analyses were confounded by the age of foliage sampled and the quantity of nitrogen that each treatment had received. After day 110, data was analysed for a particular date and was thus confounded by time since application.

**Main treatments** Fertilizer was detected on the first sampling, some 14 days after application for each treatment and Ndff continued to increase steadily thereafter (Figure 5.4a). By day 55 the similarity between the three treatments was evident regardless of the quantity of nitrogen applied (Table 5.2). When the 1982/83 foliage sampling was discontinued on November 15, a quarter of the foliar nitrogen was derived from the fertilizer for the 3-Split and 9-Split treatments. The newly expanding 1983/84 foliage on this date showed appreciably higher

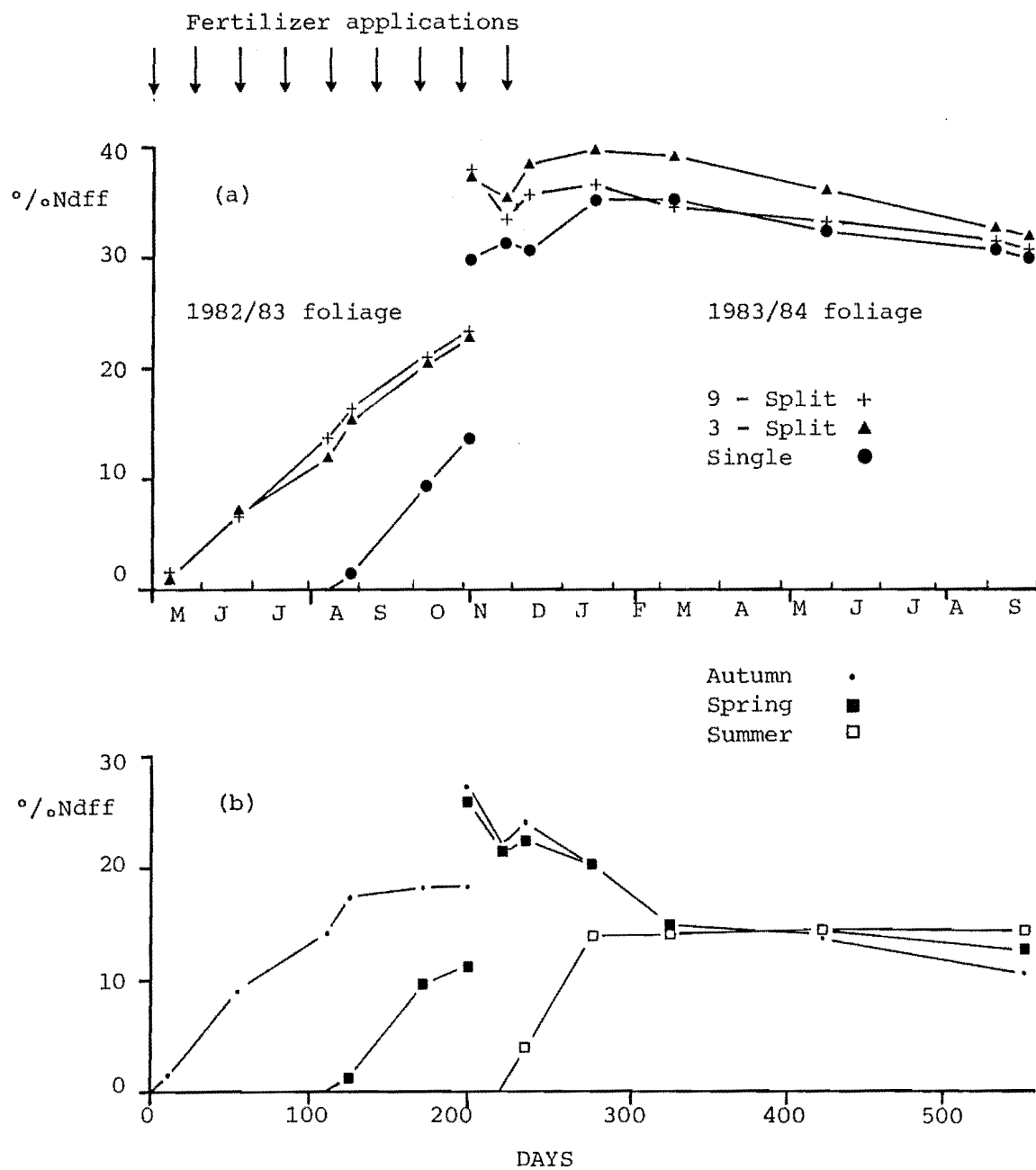


Figure 5.4 Percentage of nitrogen derived from the fertilizer (a) main treatments, (b) seasonal treatments.

labelling (Figure 5.4a). This was particularly pronounced for the Single treatment with 14 and 30% Ndff in the older and new foliage respectively. This is in agreement with several other studies (e.g. Mead and Pritchett 1975a, Melin *et al.* 1983). This may be attributed to the active physiological status of the young expanding needles with a correspondingly high demand for nitrogen. The significantly higher Ndff on the Single treatment after 110 days (Table 5.2) is because these young needles were sampled.

Table 5.2 Percentage of nitrogen derived from the fertilizer (% Ndff) for 110 days after the first applications of the main treatments.

Day since first application	Single	3-Split	9-Split	(SE)	P
	----- % Ndff -----				
14	1.4 (4/9, 90)*	1.0 (16/5, 30)	1.4 (16/5, 10)	(0.36)	0.640
55	9.4 (18/10, 90)	7.0 (27/6, 30)	6.4 (27/6, 20)	(1.97)	0.545
110	31.4# (13/12, 90)	11.8 (22/8, 30)	13.4 (22/8, 40)	(3.16)	0.003

\* : date of sampling and grams of nitrogen applied by then.

# : 1983/84 foliage.

(SE): standard error.

p : probability of differences according to ANOVA.

The labelling in the expanding 1983/84 foliage initially declined for the 3-Split and 9-Split treatments as a consequence of dilution by less enriched nitrogen. The subsequent rise coincided with the final fertilizer application on December 13. This rise peaked in February, some 7 weeks after the application. The Single treatment also peaked in February even though this application was 5 months earlier. This longer response was presumably a consequence of a heavier fertilizer application. After the summer peak there was a decline through winter and into the next growing season. There were no statistically significant ( $p < 0.2$ ) differences between the three treatments for any of the 1983/84 foliage sampling dates (analysis not presented).

Seasonal Treatments The increase in Ndff up to day 55 was the same for the Autumn and Spring treatments (Table 5.3). The new foliage again had a higher labelling, e.g. 26% as opposed to 11% for the older foliage in the Spring treatment (Figure 5.4b). This foliage age difference was the cause of the higher labelling for the Summer

treatment up to day 55 (Table 5.3).

Table 5.3 Percentage of nitrogen derived from the fertilizer (% Ndff) for 110 days after the seasonal applications.

Day since application (30 g N)	Autumn	Spring	Summer	(SE)	P
	----- % Ndff -----				
14	1.6 (16/5)*	1.3 (4/9)	3.9# (28/12)	(0.43)	0.043
55	9.0 (27/6)	9.6 (18/10)	14.3# (7/2)	(1.76)	0.207
110	14.1 (22/8)	22.1# (13/12)	14.0# (27/3)	(1.67)	0.065

\* : date of sample.

# : 1983/84 foliage.

(SE): standard error.

p : probability of differences according to ANOVA.

The Ndff in the 1982/83 foliage of the Autumn treatment reached a plateau about 120 days after application. A similar steady state level was reached for the Summer treatment after 75 days. There was an indication of a similar level on the Spring treatment after about 100 days.

The Ndff declined in the Autumn and Spring treatments in the rapidly expanding new foliage. A steady state was reached by March at the same level as for the Summer treatment. Data were not available for September 28, so the final biomass data (October 7) have been used to complete Figure 5.4b.

There were no significant differences between treatments after February (analysis not presented).

The use of Ndff as an indicator of differences in fertilizer uptake relies on trees having the same initial nitrogen content. Clearly a tree with a large nitrogen pool in its foliage will have a lower % Ndff after N-15 application than a smaller tree *ceterus paribus*. An estimate of the initial foliar nitrogen pool at the time of application is given in Table 5.4, i.e. nitrogen concentration (Chapter 4) x older foliage biomass (Chapter 3). As these different pools might affect the interpretation of results, a co-variance analysis was performed for the day 55 data. The co-variate used was the reciprocal of nitrogen content (c.f. Chapter 4). The adjusted means are given in Table 5.5.

Table 5.4 Estimated needle nitrogen pool per tree prior to the first fertilizer applications.

Treatment	Date	% N	Old foliage biomass (g)	N Pool (g)
3-Split	2 May	1.513	688	10.41
9-Split	2 May	1.585	435	6.89
Single	22 Aug.	1.705	602	10.26
Autumn	2 May	1.598	355	5.67
Spring	22 Aug.	1.734	542	9.40
Summer*	13 Dec.	1.892	496	9.38

\*: inaccurate estimate due to flushing of new foliage.

Table 5.5 Percentage of nitrogen derived from the fertilizer, 55 days after the main treatments; actual means and means adjusted for initial nitrogen pool.

	Single	3-Split	9-Split	(SE)	P
	% Ndff				
Actual	9.4	7.0	6.4	(1.97)	0.545
Adjusted	10.4	8.0	4.5	(1.95)	0.329

(SE): standard error.

p : probability of differences according to ANOVA.

The trend in Table 5.2 is accentuated but the differences remain non-significant. The difference between the 3-Split and 9-Split is in approximate agreement with the amount of nitrogen applied. The difference between the Single and Split treatments is not. The Single treatment trees may have a limited ability to utilise the larger available pool of fertilizer nitrogen, at least into the foliage during the first 55 days. It is also possible that the available nitrogen pool was rapidly reduced because of leaching (Section 5.3.2).

The seasonal treatment data were not amenable to co-variance analysis, so the results in Table 5.3 cannot easily be "corrected". The initial nitrogen pool on the Spring treatment was much larger than the Autumn treatment (Table 5.4), therefore, the indication of a similar uptake by day 55 (Table 5.3) may be misleading.

A comparison of the Spring seasonal treatment (30 g N) with the Single treatment (90 g N) is not confounded by date of application. For the first 55 days the uptake was apparently the same (Tables 5.2 and 5.3), although the different initial pools (Table 5.4) should again be



considered. Thereafter the Ndff leveled off in the older foliage and decreased in the new foliage for the Spring treatment, while it continued to climb for the Single treatment (Figures 5.4a and 5.4b).

The uptake was, apparently, reasonably constant across rates of nitrogen and season of application for at least the first 55 days. Thereafter the continued availability of fertilizer nitrogen regulates further uptake. For the main treatments ammonium levels at 20 cm depth remained above Controls until March. The decline in Ndff (Figure 5.4a) coincided with the return to Control levels in the leachates (Figure 5.2).

**5.3.3.2 Fertilizer Nitrogen Content Per Needle** Figure 5.5 shows the fertilizer nitrogen content per needle based on the N-15 analysis. This is a product of the labelled nitrogen data (previous section) and total nitrogen needle content (Chapter 4). In Figure 5.5a data for the two crown positions are shown separately for the main treatments because of the differences in needle weight (Chapter 3). There were also differences in the seasonal treatments, but, for clarity, only the upper crown data are shown in Figure 5.5b.

A statistical analysis of N-15 content per needle is given in Table 5.6 for the first 110 days after the main treatment application. As an estimate of treatment differences in tree uptake of fertilizer N, this parameter assumes an approximately equal number of needles per tree. This was clearly not the case (Chapter 3). Accordingly a co-variance analysis was used for the day 55 data to remove the effect of variable foliar masses. The co-variate was the reciprocal of old foliage biomass (c.f. Chapter 4 and the previous section).

Table 5.6 Fertilizer nitrogen content per needle for 110 days after the first applications of the main treatments.

Days since first application		Single	3-Split	9-Split	(SE)	P
		µg N-15 / needle				
14		5	3	4	(1.1)	0.336
55	actual	47	26	25	(8.5)	0.141
	adjusted	49	29	21	(8.2)	0.146
110		a	48	68	(14.4)	-

a : new foliage sampled.

(SE): standard error.

p : probability of treatment differences.

**Main Treatments** Fertilizer N accumulated in the needles of the 3-Split and 9-Split treatments from May - August during the coldest part of the year (see Figure 2.4). The rate of accumulation increased

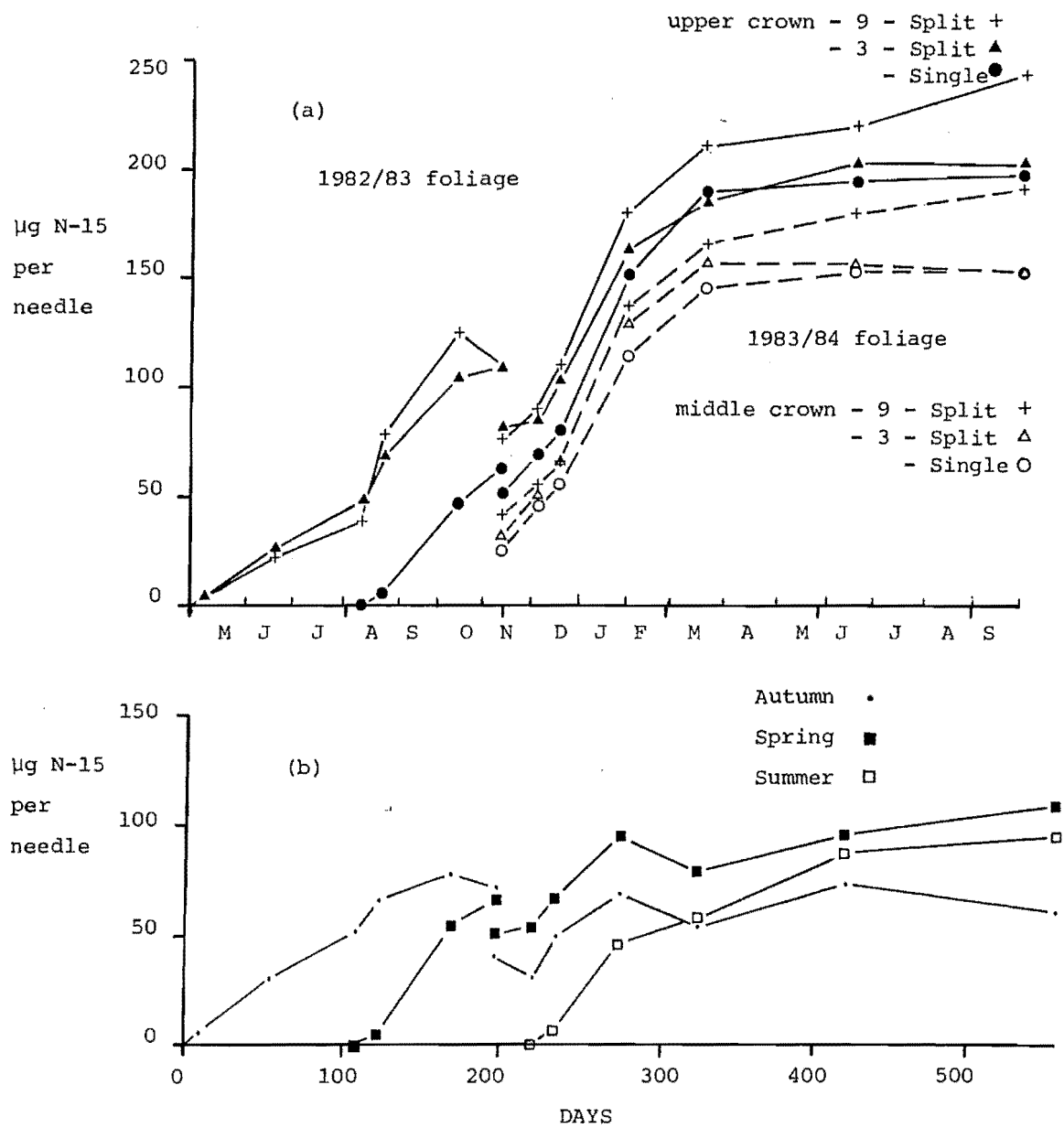


Figure 5.5 N-15 content per needle for (a) main treatments and (b) seasonal treatments

after a further application in August. From October to November there was net retranslocation of fertilizer nitrogen from the 9-Split treatment, in accordance with the trends for total nitrogen (Figure 4.2).

The adjusted means in Table 5.6 show a similar pattern to the % Ndff data in Table 5.2, i.e. an increased N-15 needle content with rate of applied nitrogen but not in proportion to the rate.

The rapid incorporation of fertilizer nitrogen into the new foliage followed the pattern of the uptake of total nitrogen (Chapter 4). By March a constant value is approached for the Single and 3-Split treatments, although during this period total nitrogen continued to increase (Chapter 4). This explains the decrease in the Ndff (Figure 5.4a). The higher N-15 contents in the 9-Split treatment are in agreement with the total nitrogen data and may again be explained by the smaller total number of needles in this treatment (Chapter 3). There were, however, no significant differences between treatments for either the upper or middle crown in Figure 5.5a.

Seasonal Treatments The uptake pattern for the Autumn treatment was initially the same as the 3-Split, but then tapered off as for the Ndff (Figure 5.5b). Similarly the Spring treatment followed the accumulation pattern of the Single treatment in the 1982/83 foliage. However, in the 1983/84 foliage the level reached was about half of that achieved for the main treatments. There were no significant differences between seasonal treatments after February (analysis not presented).

5.3.3.3 General Discussion of Foliar N-15 Uptake The major conclusions are:

- there was a similar uptake for the first 55 days regardless of season, rate or method of application,
- an apparently steady state was reached by March for all treatments,
- the fertilizer nitrogen content per needle in the main treatments was only twice that of the seasonal treatments, although three times as much nitrogen was applied.

The initial uptake figures suggest that there may be a limit to the quantity of added nitrogen the trees can utilise. Clearly, the available pool of nitrogen from a 30 g N and a 90 g N application (Spring and Single) has been utilised to the same extent by the 1982/83 foliage, i.e. approximately 65  $\mu\text{g}$  N per needle by November 15 (Figures 5.5a and b).

The data only refer to the particular foliage component sampled, so caution is required in extrapolating to a whole tree basis. For example, there may have been differences in uptake and storage into fine roots between treatments. Does Figure 5.5 indicate a cessation of fertilizer N uptake by March? Without the use of sequential tree harvests (c.f. Nambiar and Bowen 1986) this cannot be categorically

answered. However, 1983/84 foliage is a major component of the tree (Chapter 3) and is presumed to be the major site for fertilizer N accumulation (see Chapter 6). With the exception of a few autumn shoots this component of biomass was fully formed by March. Unless major retranslocation from the sampled needle biomass or fertilizer N uptake was occurring into other tree components, it may be concluded that fertilizer N uptake had virtually ceased by March. It should also be noted that there is little evidence for further uptake in the new growing season from the September 28 sample (Figure 5.5). Further evidence for this limited period of uptake (200 days after Single application) comes from the 20 cm lysimeter data showing Control ammonium levels to have been reached by March (Section 5.3.2). Other N-15 studies also support the view that uptake of fertilizer N is confined mainly or wholly to the first growing season (Mead and Pritchett 1975b, Nambiar and Bowen 1986).

The data indicate no differences in fertilizer N recovery between main or between seasonal treatments. However, the seasonal treatments have half the fertilizer nitrogen content per needle of the main treatments, but only received one third the quantity of nitrogen. Does this indicate increased uptake of fertilizer N at the lower rate? This is unlikely, given the differences in biomass response in the 1983/84 foliage (Chapter 3), i.e. 1629 g at 30 g N and 1965 g at 90 g N (Table 3.10). The relatively higher needle content for the seasonal treatments could thus be attributed to there being less foliage biomass.

5.3.3.4 Priming Effect Inorganic nitrogen fertilizers are reputed to be able to promote mineralization of native soil nitrogen. Studies using N-15 labelled fertilizers have shown mineral soil nitrogen pools to be larger than can be attributed to the fertilizer and background nitrogen alone (Popovic and Nõmmik 1972, Overrein 1972). The processes involved in this apparent priming action and its implications are, however, contested (Jansson 1971, Hauck and Bremner 1976, Laura 1975, Westerman and Tucker 1974).

Analyses are presented here to ascertain if any priming action resulted in higher foliar nitrogen levels (c.f. Heilman *et al.* 1982a). Needle nitrogen content attributed to the fertilizer on the main treatments was calculated by two methods. The direct method is based on the N-15 analyses (Figure 5.5a). The indirect method is based on the difference between total nitrogen content in Control and fertilized needles. This was calculated from the data in Table 4.8 and Figure 4.2. The N-15 data are assumed to give an estimate of actual fertilizer N uptake. A higher estimate using the indirect method would suggest a greater uptake of native soil nitrogen. Standard errors for means and differences between means were calculated according to Steel and Torrie (1981, p.143).

For the 1982/83 foliage there is some evidence for a priming effect in August for the 9-Split treatment (Table 5.7). This coincides with the highest concentration of ammonium ion in the 20 cm lysimeters

(Section 5.3.2). On most other occasions and for the 1983/84 foliage (Table 5.8), the N-15 method gives a higher estimate, which may be called a "negative priming effect" (Hauck and Bremner 1976). This is particularly pronounced from February/March onwards when fertilizer nitrogen according to the difference method declines. This is due to a relatively greater increase in needle nitrogen content on the Control trees (Figure 4.2). The difference method is clearly inappropriate where there are alternative sinks, due to greater biomass accumulation in fertilized trees.

Table 5.7 Comparison of the two methods for calculating fertilizer derived nitrogen per needle. 1982/83 foliage.

Sampling date	Method	Single	3-Split	9-Split	(SE)
--- µg fertilizer N / needle ---					
May 16	DIFF	39	-36	1	(45)
	N-15	0	3	4	(1)
June 27	DIFF	13	-1	21	(39)
	N-15	0	26	25	(9)
August 22	DIFF	29	47	120	(54)
	N-15	0	48	38	(11)
September 4	DIFF	-18	35	97	(44)
	N-15	5	69	78	(18)
October 18	DIFF	66	62	151	(54)
	N-15	47	104	126	(22)
November 15	DIFF	73	70	90	(59)
	N-15	62	109	110	(19)

(SE): standard error.

DIFF: fertilizer nitrogen calculated by difference between fertilized and Control needles.

N-15: fertilizer nitrogen calculated by N-15 method.

Table 5.8 Comparison of the two methods for calculating fertilizer derived nitrogen per needle. 1983/84 foliage, upper crown.\*

Sampling date	Method	Single --- $\mu\text{g}$ fertilizer N / needle ---	3-Split	9-Split	(SE)
November 15	DIFF	28	64	57	(24)
	N-15	51	81	78	(13)
December 13	DIFF	31	48	86	(37)
	N-15	69	85	92	(16)
December 28	DIFF	58	50	79	(35)
	N-15	79	103	110	(17)
February 7	DIFF	100	65	141	(30)
	N-15	150	162	180	(23)
March 27	DIFF	31	-43	123	(34)
	N-15	188	184	210	(21)
June 20	DIFF	18	-28	68	(49)
	N-15	193	203	220	(22)
September 28	DIFF	-12	-85	89	(80)
	N-15	197	203	247	(20)

(SE): standard error.

DIFF: fertilizer nitrogen calculated by difference between fertilized and Control needles.

N-15: fertilizer nitrogen calculated by N-15 method.

\* : the trends were similar for the middle crown needles.

Clearly the analysis is encumbered by highly variable data. Two approaches were tried to lessen this effect:

- (i) an analysis of co-variance to remove the effect of different initial needle nitrogen contents. The data for May 2 was used as the co-variate.
- (ii) repeating the analysis for needle nitrogen concentration only (c.f. Heilman *et al.* 1982a) to reduce the variability inherent in using the composite parameter, needle nitrogen content.

The analysis using needle nitrogen concentration for the 1982/83 foliage gave the same patterns as for nitrogen content. Data for one date, August 22, are presented in Table 5.9 along with the original and adjusted nitrogen contents. The means have been adjusted but the increase in precision is negligible.

Table 5.9 Comparison of fertilizer derived nitrogen calculated by the N-15 and difference methods for the August 22 foliage sample.

		Single	3-Split	9-Split	(SE)
µg N / needle (Table 5.7)	DIFF	29	47	120	(54)
	N-15	0	48	38	(11)
adjusted µg N per needle increase	DIFF	8	64	113	(53)
	N-15	0	48	388	(11)
% N increase	DIFF	0.06	0.24	0.42	(0.111)
	N-15	0	0.15	0.28	(0.070)

(SE): standard error.

DIFF, N-15: as for Table 5.8.

The indication of a priming effect in August following autumn and winter applications is in agreement with Heilmen *et al.* (1982a) as is the reverse trend in subsequent samplings. Hauck and Bremner (1976) observe that the difference between the two methods is usually greatest during the early harvests of a sequential harvest experiment (e.g. Westerman and Kurtz 1973). However, the priming effect, even if real, is only transitory and the subsequent reverse effect is as important. This indicates that on fertilized plots the uptake of native soil nitrogen is depressed. This apparent negative priming effect has been observed previously (Gadet and Soubies 1966, Westerman and Tucker 1974). The latter authors observed this effect when high concentrations of salts were added along with ammonium. This decreased activities of microorganisms with an attendant slowing of the mineralization rate and also the immobilization of added ammonium. It is unlikely that the quantities or concentration of applied nitrogen in this study adversely affected the soil microflora. Indeed the first observed effect of urea applications was an algal bloom on the soil surface. This initial reaction could have been associated with the possible priming action discussed above.

If the mineralization/immobilization processes were not adversely affected then it may be assumed that a similar pool of native soil nitrogen is available on fertilized and control plots. Why do fertilized trees apparently not use this pool? Or, conversely, why is there a preferential uptake of N-15? Biological discrimination between the two isotopes of nitrogen is possible (Bremner *et al.* 1966), but this can generally be discounted in most experimental situations (Jansson 1971, Hauck and Bremner 1976). It is more likely that the trees could more readily utilise the available pool of labelled

fertilizer nitrogen which "bathed" the roots. Ammonium from mineralization of native soil nitrogen would first have to move to the root surface.

The difference in the uptake of fertilizer N estimated by these two methods is discussed further in Chapter 6, following presentation of the total tree uptake figures at the end of the experiment.

5.3.3.5 Fertilizer N Gradients Within the Crown In the previous chapter, gradients in total needle nitrogen concentration were discussed. Here an analysis of fertilizer gradients is presented using the ratio of upper to middle crown atom-% N-15, (Table 5.10 and 5.11).

Table 5.10 Upper:Middle crown ratio of atom % N-15 for the main treatments.

Date	Single	3-Split	9-Split	(SE)	P
15 Nov.	0.93	1.00	0.97	(0.019)	0.032
13 Dec.	0.94*	1.04	0.99	(0.036)	0.165
28 Dec.	0.93	0.98	1.00	(0.018)	0.030
7 Feb.	0.96	0.99	1.00	(0.016)	0.111
27 Mar.	0.97	0.98	1.00	(0.015)	0.186
20 Jun.	0.98	1.00	0.99	(0.010)	0.135
28 Sept.	0.95	1.00	1.00	(0.008)	0.029

\* : 3 replicates only.

(SE): standard error.

p : probability of differences between Single and Split applications according to single degree of freedom contrasts in ANOVA.

Table 5.11 Upper:Middle crown ratio of atom % N-15 for the seasonal treatments.

Date	Autumn	Spring	Summer	(SE)	P
15 Nov.	0.99	1.00	no fertilizer	(0.037)	-
13 Dec.	0.96	0.97	no fertilizer	(0.001)	-
28 Dec.	1.04	1.08	0.96	(0.018)	0.048
7 Feb.	1.08	0.98	0.93	(0.024)	0.089
27 Mar.	1.01	1.00	0.97	(0.022)	0.323
20 Jun.	1.01	0.99	0.98	(0.007)	0.143

(SE): standard error.

p : probability of treatment differences between Summer and the other two treatments according to single degree of freedom contrasts in ANOVA.



The differences between positions were small, i.e. the ratio approached 1.0. The Summer treatment (Table 5.11) shows a consistent gradient with a higher enrichment in the middle crown which was more pronounced just after the fertilizer was applied. Similarly the Single treatment (Table 5.10) shows a consistent gradient favouring the middle crown whereas the Split treatments show no gradient.

There is some controversy in the literature (c.f. Melin *et al* 1983) as to whether the concentration of labelled nitrogen increases or decreases with height in the crown. Worsnop and Will (1980) found an increase from the base to the top of 14-year-old radiata pine. Nambiar and Bowen (1986), working with younger trees of the same species, found no gradient in N-15 enrichment with tree height. Mead and Pritchett (1975a) showed the highest concentrations of labelled nitrogen to be in the lower crown of slash pine as do Bjorkman *et al.* (1967) in young scots pine. These apparently mutually inconsistent results might be explained by factors which regulate translocation and redistribution of nitrogen within the tree (Melin *et al.* 1983).

Definite gradients in total nitrogen concentrations were observed early in the season which were related to the preferential growth of upper crown needles at this stage (Chapter 4). Clearly, the N-15 enrichment does not follow this pattern. Either there is an opposite gradient, apparently related to time since fertilization, or none at all. Mead and Pritchett (1975a) explain their gradient as labelled nitrogen becoming further diluted with internally cycling nitrogen (natural enrichment) as it moves up the crown. In this study, there is some retranslocation from older foliage (Chapter 4). In the case of the Summer treatment any retranslocated nitrogen early in the season will be at a natural enrichment. If it moves preferentially to the upper crown, dilution of labelled nitrogen will occur resulting in the gradient seen (Table 5.11). In the case of other treatments retranslocated nitrogen from the 1982/83 needles would itself be labelled, but at a lower enrichment, which would still support the dilution hypothesis. This may explain the gradient in the Single treatment where any retranslocated nitrogen from 1982/83 needles has a lower enrichment compared with the 3-Split and 9-Split treatments (Figure 5.4a).

Bjorkman *et al.* (1967) show a parallel gradient for total nitrogen and atom % N-15. Mead and Pritchett (1975a) show no gradient for total nitrogen, indicating a uniform demand. In this study, there was a very definite gradient early in the season for total nitrogen. The absence of a fertilizer N gradient to support this either suggests discrimination between nitrogen isotopes (c.f. Shearer *et al.* 1980, Kohl *et al.* 1979) or retranslocation of lower enriched nitrogen to the upper crown; a dilution hypothesis. An alternative explanation which may be applicable soon after fertilization, is a time lag for labelled nitrogen to reach the upper crown.

The formation of autumn shoots was noted from late February

(Chapter 3). These were sampled after elongation was complete and were analysed for N-15 on June 20. The Ndff was the same as the main flush (data not presented). The even labelling indicates that nitrogen from the main flush was retranslocated to the autumn shoots. Fife and Nambiar (1984) found that retranslocation of nitrogen from spring needles was reduced when summer shoots were removed. They contended that the nitrogen requirements of summer shoots were met by retranslocation from spring needles.

#### 5.3.4 Soil Analyses

5.3.4.1 pH A small increase in pH following urea application was apparent (Table 5.12). Worsnop and Will (1980) report changes in soil pH did not exceed 0.5 of one unit following an application of 200 kg N/ha as a urea solution. The decrease in acidity with depth agrees with the usual trend (Fritchett 1979).

Table 5.12 pH changes following the Single application of fertilizer.

Depth (cm)	Months after fertilizer application				
	0	2	4	6	10
0-10	4.67*(0.099)	4.90(0.123)	4.78(0.048)	4.83(0.098)	4.78(0.100)
10-30	4.82 (0.298)	5.06(0.114)	4.86(0.108)	4.96(0.038)	4.99(0.026)

\*: mean of four plots and standard error.

5.3.4.2 Nitrogen The total nitrogen concentrations were highly variable (Appendix 11). The means for all plots and dates were 0.084% N for 0-10 cm and 0.018% N for 10-30 cm.

The N-15 results (Appendix 11) are summarised in Table 5.13 as the percentage of nitrogen derived from the fertilizer. Within two

Table 5.13 Percentage of nitrogen derived from the fertilizer in soil nitrogen for 14 months following fertilization: Single treatment only.

Depth (cm)	Months after fertilizer application				
	2	4	6	10	14*
	----- % Ndff -----				
0-10	5.8#(1.01)	2.7(0.97)	6.0(2.44)	3.7(0.92)	2.8(0.63)
10-30	6.0 (1.86)	2.4(1.08)	3.1(0.50)	2.8(0.55)	1.7(0.35)

\*: final soil sampling (Oct. 1984) from Chapter 7.

#: mean of four plots and standard error.

months of application there was an even labelling of nitrogen at both depths. This declined from two to four months and then remained fairly constant with the exception of the apparently anomalous data after six months.

The recovery of fertilizer nitrogen was calculated from the N-15 and % N data utilising the bulk densities in Chapter 7. The data for 0-10 cm at six months (Table 5.14) are probably in error. The recovery for one plot was clearly too high, apparently due to an extreme N-15 atom % value (Appendix 11).

Table 5.14 Recovery of applied fertilizer in the soil for 14 months following fertilization. Single treatment only.

Depth (cm)	Months after fertilizer application				
	2	4	6	10	14*
	----- % of applied -----				
0-10	42.2#(7.62)	21.6(7.72)	38.6(18.04)	22.2(4.45)	20.9(4.73)
10-30	22.5 (7.84)	9.1(3.80)	12.4 (1.88)	8.8(1.83)	9.6(2.06)
Sum	64.7(15.19)	30.7(11.05)	51.1(18.02)	31.0(6.08)	30.5(6.70)

#: final soil sampling (Oct. 1984) from Chapter 7.

#: mean of four plots and standard error.

Twice as much fertilizer N was recovered in the upper horizon (Table 5.14). After two months 65% of the fertilizer N was accounted for in the top 30 cm of soil. This halved during the next two months, presumably as tree uptake and leaching occurred (Figures 5.2 and 5.5a).

Within four months the recovery of fertilizer N to 30 cm depth had stabilised at 30%. This suggests that an equilibrium between applied N-15 and native soil nitrogen had been reached. The major period of "loss" from the sampled soil was apparently within four months of application. The peaks of ammonium ion concentration in leachates occurred within two months of application at 20 and 40 cm depth (Figure 5.2)

Other studies have also shown an initial period of leaching followed by stable soil N-15 levels after a few months. Mead and Fritchett (1975a) show equilibrium conditions within 12 weeks following applications of ammonium sulphate to a slash pine ecosystem. Popovic and Nõmmik (1972) showed urea nitrogen to have reached an equilibrium phase within 6 weeks of application to soil under norway spruce.

5.3.4.3 Fine Roots The seasonal pattern of nitrogen concentration was the same for both treatments studied (Figure 5.6a). The initial rise was after a fertilizer application in the spring.

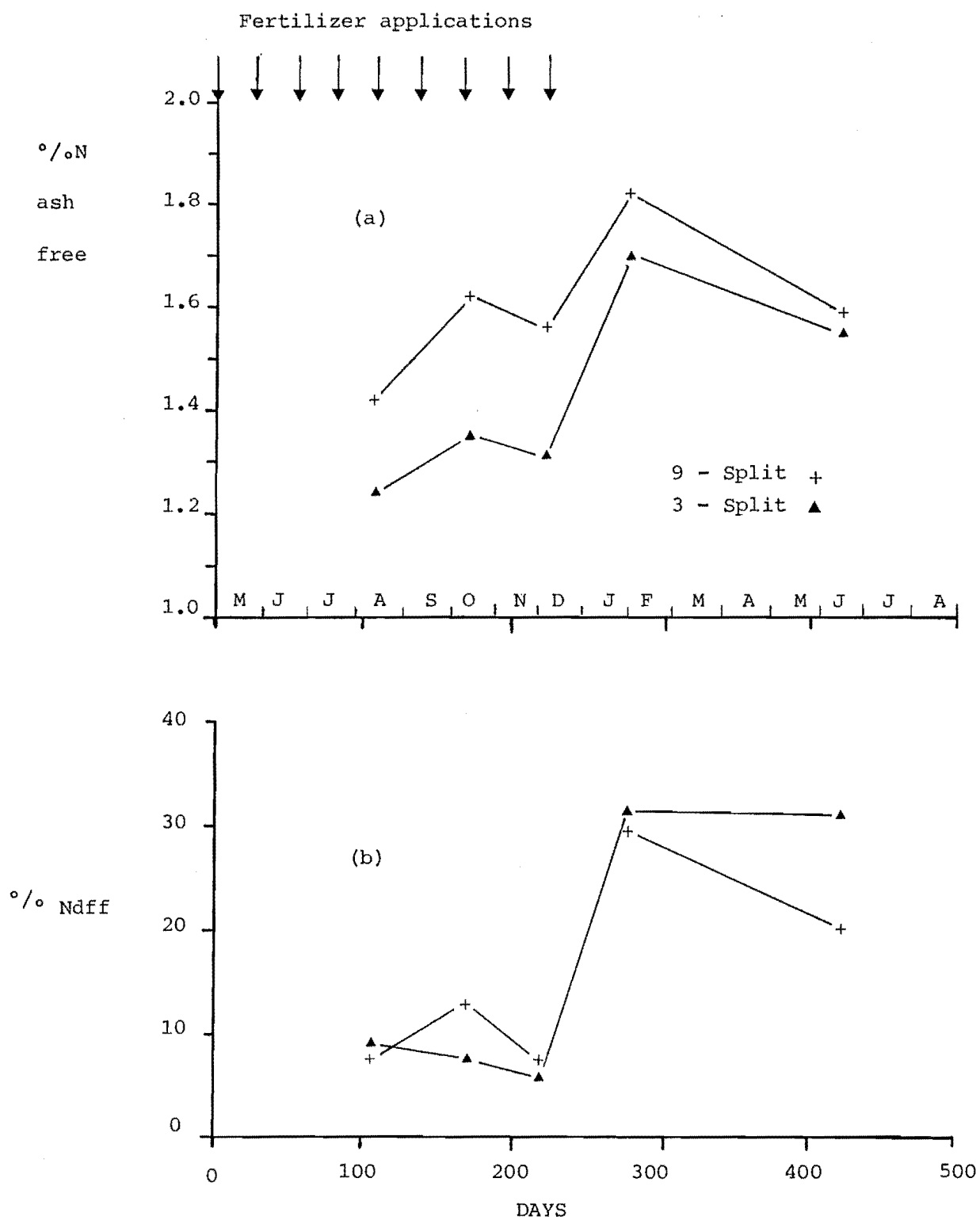


Figure 5.6 Nitrogen status of fine roots  
(a) concentration, (b) percentage of  
nitrogen derived from fertilizer

Foliar %N also rose from August to October (Figure 4.1). The decline in %N from October to November coincided with retranslocation of foliar nitrogen to the newly expanding foliage. The rise from December to February appeared to correspond with the major phase of nitrogen uptake into the new foliage (Figure 4.2). The decline from February to June occurred when accumulation in the foliage was declining (Figure 4.2).

The proportion of nitrogen derived from the fertilizer broadly followed the same pattern as for total nitrogen (Figure 5.6b). The large increase from December to February indicated a major phase of fertilizer N uptake. From February the steady or declining value could indicate a cessation of fertilizer N uptake (c.f. Section 5.3.3.1).

There are few published accounts of seasonal variation in the nitrogen status of roots. The lack of data for Control trees may preclude comparisons with other studies. However, the seasonal patterns of foliar nitrogen concentration were similar between fertilized and Control trees (Figure 4.1). McClaugherty *et al.* (1982) report an increased % N in spring and autumn with a decline in winter for 53-year-old red pine (*Pinus resinosa* Ait) in Massachusetts. This does not agree well with Figure 5.6a, although August - October could be a spring increase. Clinton (1986), working with 14-year-old radiata pine in New Zealand, sampled fine roots through one year. % N was similar for most dates, except for a significant ( $p < 0.05$ ) peak in August.

Increases in % N probably represent an influx of nitrogen into fine roots from fertilizer or mineralized soil nitrogen. Decreases may be attributed to retranslocation of nitrogen from fine roots to other tree components or a significant biomass increase of fine roots, causing a dilution effect.

A depletion of fine root nitrogen between October and December in response to demands from the newly expanding foliage seemed possible. Roots may act as storage sites for nitrogen during winter (van den Driessche 1984). This has been shown for fruit trees (Taylor and May 1967) with some indication for douglas fir (van den Driessche and Webber 1977).

The decline in % N from February to June could be retranslocation, but the major demand for nitrogen above ground is declining at this time (Chapters 3 and 4). It is more likely that the substantial biomass response in fine roots occurred during this period. The lower nitrogen concentrations could then be due to a dilution effect. The steady or declining Ndff values suggest that the nitrogen source was now at a low or natural enrichment. This would be so, if the N-15 (last applied on December 13) had come to equilibrium with the soil nitrogen (c.f. Section 5.3.4.2).

#### 5.4 CONCLUSION

There are three indicators of the length of time a freely available N-15 pool exists for tree uptake:

- (i) leaching data,
- (ii) foliar N-15 content,
- (iii) soil sampling.

The leaching data suggest that there were initially high levels of ammonium ion in solution. These decreased until Control levels were reached about March, 200 days after the Single application. The concentration detected for much of this period was only 2-4 times higher than the Control at 0.1 ppm. The absolute size of the N-15 pool cannot, however, be calculated from the leaching data.

The soil sampling for the Single treatment indicates that the total N-15 pool came to equilibrium with the soil nitrogen pool within 120 days. The leachate ammonium levels beyond this date may represent only a small amount of N-15 available for uptake.

Probably the best indicator of fertilizer N availability within the soil is the foliar N-15 levels. These show rapid accumulation from November to February (Figure 5.5), which suggests that there is an available N-15 pool in the soil. This is supported by the leaching data, but apparently contradicted by the soil sampling, which suggests equilibrium conditions by December. It is, however, probable that total N-15 analyses are not an appropriate means to determine availability of fertilizer N. Alternatively, the increase in foliar N-15 could be due to retranslocation from the roots rather than uptake for the soil. This seems unlikely, given the increase in Ndff in fine root at this time (Figure 5.6b). After March, N-15 uptake into the foliage virtually ceased, although total nitrogen continued to rise (Chapter 4). There is also evidence for a continued demand for nitrogen by other tree components after March (Chapters 3 and 4), in particular the fine roots.

It is suggested that by March there was no longer an N-15 pool available for tree uptake. This does not mean that nitrogen uptake ceased, but rather that uptake was from the native soil nitrogen pool, albeit slightly labelled with immobilized N-15.

## CHAPTER 6

## TREE UPTAKE AND DISTRIBUTION OF NITROGEN

## 6.1 INTRODUCTION

The simplest method of accounting for the fertilizer nitrogen taken up by trees is the difference between nitrogen content in fertilized and unfertilized trees. The quantity taken up is often small in relation to the total nitrogen content thus encumbering the method with a relatively large experimental error (Melin *et al.* 1983). It also assumes that all the extra nitrogen comes from the fertilizer. This assumption may not be valid if fertilizer enhances availability of native soil nitrogen which is known as a priming effect (Chapter 5). Misleadingly high recoveries may also be a result of a biomass response on fertilized trees, enabling a much greater volume of soil to be exploited. Studies where dramatic responses to fertilizer occur will require particular care in interpretation (e.g. Waring 1969, 1980, Ballard 1978). The difference method may require large numbers of trees to overcome inherent variability in tree nitrogen contents. The onus is also on the investigator to achieve high levels of accuracy and precision in the total nitrogen analysis. Table 6.1 gives the recovery of applied nitrogen by this method for several studies.

Table 6.1 Recovery of applied nitrogen in pines--the difference method.

Species	Age and response period (years)	Fertilizer	Rate (kg N/ha)	Tree recovery (%)	Reference
radiata pine	6 (1)	$(\text{NH}_4)_2\text{SO}_4$	300	10	Oliver 1979
radiata pine	0 (3)	$\text{CO}(\text{NH}_2)_2$	230	14*	Nielsen <i>et al.</i> 1984
radiata pine	0 (5.5)	$\text{CO}(\text{NH}_2)_2$	500	29	Waring 1969
radiata pine	0 (7)	$\text{CO}(\text{NH}_2)_2$	700	41	Waring 1980
radiata pine	0 (3)	$\text{CO}(\text{NH}_2)_2$	130	35	Ballard 1978
loblolly pine	4 (2)	$\text{NH}_4\text{NO}_3$	224	3	Baker <i>et al.</i> 1974
	4 (2)	$\text{NH}_4\text{NO}_3$	112+112	7	
corscian pine	36 (3)	$(\text{NH}_4)_2\text{SO}_4$	252	51*	Miller <i>et al.</i> 1976

\* including roots.

To overcome the above difficulties, N-15 labelled fertilizers have been used (Nõmmik 1966, Mead and Pritchett 1975b, Melin *et al.* 1983, Heilman *et al.* 1982b, Nambiar and Bowen 1986). This method relies only on being able to detect a portion of the applied tracer in a

chemically defineable state (Hauck and Bremner 1976). These authors review tracer and non-tracer methods and conclude that the former are more accurate. However, certain assumptions have to be made, the main one being that biological interchange of labelled nitrogen with unlabelled soil nitrogen is not confounding the results. Table 6.2 gives tree recoveries for several studies based on N-15 data. Recovery has usually been less than 20%, but recent studies (Heilman *et al.* 1982b and Nömmik pers. comm.) have shown higher figures.

Table 6.2 Recovery of applied nitrogen in forest trees -- the N-15 method.

Species	Age and response time (years)	Fertilizer	Rate (kg N/ha)	Tree recovery (%)	Reference
scots pine	12 (2)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	50	*3-8	Nömmik 1966
scots pine	15 (1)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	60	15	Bjorkman <i>et al.</i> 1967
scots pine	80 (1)	CO(NH <sub>2</sub> ) <sub>2</sub>	53	2	Paavilainen 1973
scots pine	130 (2)	NH <sub>4</sub> NO <sub>3</sub>	100	12-28	Melin <i>et al.</i> 1983
scots pine	35 (2)	NH <sub>4</sub> NO <sub>3</sub>	150	45	Nömmik pers. comm.
and norway spruce	50 (2)	NH <sub>4</sub> NO <sub>3</sub>	150	33	
	120 (2)	NH <sub>4</sub> NO <sub>3</sub>	150	20	
	50 (2)	CO(NH <sub>2</sub> ) <sub>2</sub>	150	20	
slash pine	13 (2)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	56	#11	Mead and Pritchett
			224	11	1975b
radiata pine	0.1(1)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	8	6	Nambiar and Bowen
	1		34	18	1986
douglas fir	6 (2)	CO(NH <sub>2</sub> ) <sub>2</sub>	224	30	Heilman <i>et al.</i> 1982b
douglas fir	4 (2)	CO(NH <sub>2</sub> ) <sub>2</sub>	200	+42	Pang 1985
		NH <sub>4</sub> NO <sub>3</sub>	200	66	

\* : above ground only.

# : trees not root isolated.

+ : pot study.

## 6.2 METHODS

### 6.2.1 Subsampling

After oven drying, the tree components designated in Chapter 3 were carefully subsampled to obtain a small sample for chemical analysis.

Coarse roots, 1-year and older twigs were ground to sawdust in a hammer mill. A subsample taken using a divider was then finely ground



in a ring and puck mill. Samples of 1-year stem from three trees were also prepared in this manner. These were analysed to confirm that their nitrogen status could be estimated from 1-year twig data.

Foliage samples were spread on trays and samples of fascicles picked out at random and ground in a ring and puck mill.

Wood discs were split with an axe, roughly ground in a Wiley mill, then finely ground in a ring and puck mill. Bark samples were directly ground in the latter. Current twigs were ground in a Wiley mill, then a subsample was taken for fine grinding.

The rootstock was not sampled. Its nitrogen concentration was assumed to be similar to the lower stem, i.e. a weighted mean of % N for stem wood and bark. The atom % N-15 was taken to be equal to coarse roots. Even if false, these assumptions are unlikely to result in serious errors, since the nitrogen content of the rootstock is low in relation to that of the rest of the tree (c.f. Melin *et al.* 1983).

All the above procedures were carried out with great care to avoid cross contamination. Unfertilized trees were always sampled first and grinders were thoroughly cleaned between samples.

#### 6.2.2 Chemical Analysis: Total Nitrogen

200 mg of finely ground material was taken from each tree component for Kjeldahl nitrogen analysis as in Chapter 4. All samples were run in duplicate and a precision level of 3% set, i.e. the percentage difference between duplicates relative to their mean is <3%. The accuracy of analysis was checked by running the 1985 IUFRO interlaboratory comparison samples with all batches. The agreement with the international values is excellent (Table 6.3). Will (1986) discusses the results from the comparison.

Table 6.3 Nitrogen results for 1985 IUFRO interlaboratory comparison.

Sample	% N oven dry weight		
	Thomas	International	
	Lab 51 (sd)	Mean (sd)	Median (mad)
* -----			
85/1 tulip poplar foliage ( <i>Liriodendron tulipifera</i> )	1.79 (0.021)	1.72 (0.09)	1.74 (0.05)
85/2 douglas fir foliage	1.22 (0.012)	1.16 (0.07)	1.18 (0.03)
85/3 radiata pine foliage	1.37 (0.018)	1.34 (0.08)	1.36 (0.05)
85/4 radiata pine wood	0.09 (0.004)	0.12 (0.08)	0.10 (0.01)
85/5 radiata pine bark	0.34 (0.005)	0.33 (0.04)	0.34 (0.02)
85/6 radiata pine litter	0.67 (0.007)	0.65 (0.09)	0.67 (0.03)
-----			

\*: mean of four, except 85/3 and 85/5, n=17, 85/4, n=11.

### 6.2.3 Chemical Analysis: N-15

After titration to determine total nitrogen, the samples were acidulated and evaporated to dryness (Section 5.2.3). The duplicate wood samples were analysed for N-15 separately. The other duplicates were combined.

Aliquots containing 200-300 µg N were transferred to vials and sent to Waikato University for isotope ratio analysis.

### 6.2.4 Calculations

The total nitrogen results are presented as nitrogen concentration on an oven dry weight basis with the exception of fine roots which are on an ash free basis.

The percentage recovery of applied fertilizer for each tree component can be calculated from

$$\frac{\text{nitrogen content} \times \text{percentage derived from fertilizer}}{\text{nitrogen applied}}$$

or expanded

$$\frac{\% \text{ N} \times \text{biomass (g)} \times 100 (c-b)}{\text{wt N applied (g)} (a-b)}$$

where a, b, and c are atom % N-15 for the fertilizer, unfertilized and fertilized samples respectively. The weight of nitrogen applied is 90.16 and 30.05 grams for main and seasonal treatments respectively.

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 Total Nitrogen Concentration

The general pattern was for trees fertilized with 90 g N to have slightly lower concentrations due to the dilution effect caused by the growth response (Table 6.4). The smaller 9-Split trees accordingly have slightly higher values. However, none of the differences between treatments was significant ( $p < 0.05$ ). Similarly the seasonal treatments show no significant differences with the exception of fine roots (Table 6.5). The Autumn treatment had a significantly higher ( $p < 0.05$ ) nitrogen concentration in the fine roots than the other two treatments. This may be explained by the biomass of Autumn fine roots

being only 537 g, compared with 1073 g and 953 g for Spring and Summer treatments respectively (Table 3.7, Chapter 3).

Table 6.4 Nitrogen concentration in tree components at the end of the experiment (Oct. 1984) for the main treatments.

Tree component	Control	Single	3-Split	9-Split	(SE)	P
	----- % oven dry weight -----					
Foliage						
current	2.02	1.82	1.84	1.85	(0.072)	0.227
1-year	1.60	1.50	1.46	1.62	(0.059)	0.169
older	1.16	1.11	1.11	1.20	(0.046)	0.421
Twigs						
current	1.56	1.47	1.47	1.53	(0.072)	0.770
1-year	0.52	0.47	0.48	0.52	(0.030)	0.563
older	0.33	0.29	0.27	0.29	(0.019)	0.300
Stem						
-lower, wood	0.17	0.15	0.15	0.17	(0.009)	0.344
bark	0.62	0.61	0.60	0.69	(0.031)	0.241
Roots						
coarse	0.33	0.31	0.29	0.29	(0.018)	0.467
fine*	1.03	0.99	0.91	0.95	(0.056)	0.454

\* : concentration expressed on an ash free basis.

(SE): standard error.

p : probability of treatment differences according to ANOVA.

Concentrations decrease with increasing age of foliage and twigs. These concentrations are similar to published values for radiata pine (Stewart *et al.* 1981), although for woody components they tend to lie at the upper end of the range because of the young age of the trees (Madgwick *et al.* 1977). There is a dearth of information on nutrient concentrations in radiata pine roots (Madgwick 1985). In an early study Will (1986) reported 0.22% N for roots <3 mm. Recently Clinton (1986) has given a value of 0.45% N for roots <2 mm. These two studies were in 18 and 14-year-old stands respectively. The higher values reported in Table 6.4 may be a result of stand age, methodology, time of sampling, or site fertility. Nambiar (1984b) cites unpublished data showing concentrations of 1.15 - 1.35% N in fine roots of 12-year-old radiata pine in autumn. These and the values reported above are in reasonable agreement with published data for a variety of tree species (see Kimmins and Hawkes 1978).

Table 6.5 Nitrogen concentration in tree components at the end of the experiment (Oct. 1984) for seasonal treatments.

Tree component	Control*	Autumn	Spring	Summer	(SE)	P
	----- % oven dry weight -----					
Foliage						
current	2.02	1.99	1.95	1.93	(0.071)	0.862
1-year	1.60	1.70	1.54	1.54	(0.074)	0.367
older	1.16	1.23	1.09	1.07	(0.080)	0.425
Twigs						
current	1.56	1.64	1.52	1.73	(0.130)	0.587
1-year	0.52	0.52	0.45	0.49	(0.019)	0.188
older	0.33	0.33	0.30	0.29	(0.026)	0.645
Stem						
lower, wood	0.17	0.18	0.16	0.15	(0.007)	0.158
bark	0.62	0.64	0.60	0.59	(0.043)	0.731
Roots						
coarse	0.33	0.36	0.34	0.31	(0.010)	0.116
fine #	1.08	1.20	1.02	1.05	(0.031)	0.049

\* : Control, mean of four, reproduced for comparison.

# : concentration expressed on an ash free basis.

(SE): standard error.

p : probability of treatment differences according to ANOVA.

### 6.3.2 Total Nitrogen Content

Analyses of variance between nitrogen content in all tree components generally showed no significant differences (Table 6.6 and 6.7). This was not surprising, given the variability evident in the data. Accordingly a co-variance analysis was run, using initial D<sup>2</sup>H to remove the effect of tree size and, by implication, initial nitrogen content. For this analysis the seasonal treatments were pooled which was deemed valid given the non-significance in Table 6.7. The adjusted mean nitrogen content for selected tree components is given in Table 6.8. It was interesting to note from the co-variance analysis that 68% of the variation in above ground nitrogen content was explained by the regression with D<sup>2</sup>H. In contrast only 2% of the below ground variation was explained. This supports the contention in Chapter 3 that shoot growth is to a large extent dependent on initial tree size whereas roots are not. Clearly the use of co-variance analysis for below ground components was inappropriate but was performed to maintain comparability.

Table 6.6 Nitrogen content in tree components at the end of the experiment (Oct. 1984) for the main treatments.

	Control	Single	3-Split	9-Split	(SE)	P
	g N					
Foliage						
current	3.1	2.8	2.8	2.5	(0.45)	0.833
1-year	25.1	29.9	31.8	27.6	(2.51)	0.308
older	6.5	6.6	7.5	5.1	(0.58)	0.081
Twigs						
current	3.0	3.1	3.4	3.1	(0.27)	0.670
1-year	3.7	4.4	4.4	4.6	(0.53)	0.657
older	2.4	2.8	2.8	2.3	(0.51)	0.911
Stem						
1-year*	1.2	1.1	1.6	1.5	(0.23)	0.370
wood	2.5	2.5	2.7	2.6	(0.23)	0.812
bark	1.8	2.1	2.0	2.3	(0.19)	0.529
Above ground						
	49.3	55.3	59.0	51.7	(4.42)	0.456
Roots						
rootstock#	2.0	2.6	2.1	2.9	(0.23)	0.069
coarse	3.2	4.9	4.5	4.5	(0.48)	0.126
fine	7.8	14.3	15.8	12.1	(1.37)	0.007
Below ground						
	13.0	21.8	22.4	19.5	(1.59)	0.005
TOTAL	62.4	77.1	81.7	71.2	(4.97)	0.086

(SE): standard error.

p : probability of treatment differences according to ANOVA.

\* : estimated using % N value from 1-year twigs.

# : estimated using weighted mean of % N for stem wood and bark.

There was a marked increase in total nitrogen content for the main treatments which was particularly pronounced below ground (Table 6.8). The seasonal treatments only showed an increase below ground in accordance with their biomass response (Chapter 3). There was a large pool of nitrogen in the fine roots, with the main treatments having about twice the content of Controls. The major site for nitrogen accumulation above ground was in the 1-year foliage; about 40% of total tree nitrogen occurred there.

Table 6.7 Nitrogen content in tree components at the end of the experiment (Oct. 1984) for seasonal treatments.

	Control <sup>1</sup>	Autumn	Spring	Summer	(SE)	P
	g N					
<hr/>						
Foliage						
current	3.1	2.9	3.5	1.7	(0.67)	0.298
1-year	25.1	21.6	28.8	22.3	(4.49)	0.529
older	6.5	4.4	5.9	5.1	(1.12)	0.649
Twigs						
current	3.0	3.5	3.4	2.1	(0.88)	0.124
1-year	3.7	3.3	3.0	2.9	(0.44)	0.800
older	2.4	2.1	2.3	2.3	(0.70)	0.970
Stem						
1-year*	1.2	0.8	1.3	0.5	(0.27)	0.276
lower wood	2.5	2.0	2.2	1.7	(0.17)	0.274
bark	1.8	1.8	1.6	1.2	(0.16)	0.332
-----	-----	-----	-----	-----	-----	-----
Above ground	49.3	42.5	52.3	40.0	(7.18)	0.520
-----	-----	-----	-----	-----	-----	-----
Roots						
rootstock#	2.0	1.8	2.0	1.6	(0.39)	0.769
coarse	3.2	3.5	4.6	3.8	(0.86)	0.680
fine	7.8	7.9	12.1	11.5	(2.13)	0.428
-----	-----	-----	-----	-----	-----	-----
Below ground	13.0	13.2	18.8	16.9	(2.88)	0.475
-----	-----	-----	-----	-----	-----	-----
TOTAL	62.4	55.7	71.1	56.9	(10.01)	0.553

(SE): standard error.

p : probability of treatment differences according to ANOVA.

<sup>1</sup> : control reproduced for comparison.

\*,#, : as in Table 6.6.

Table 6.8 Nitrogen contents in selected tree components, adjusted for initial tree size differences.

Tree component	Main treatments				(SE)*	Seasonal treatments	(SE)#	P
	Control	Single	3-Split	9-Split				
	g N							
TOTAL	61.38	76.10	76.88	74.35	(3.35)	64.92	(2.73)	0.010
Above ground	48.49	54.47	55.05	54.39	(2.59)	48.08	(2.11)	0.139
Below ground	12.89	21.63	21.83	19.96	(1.65)	16.84	(1.34)	0.006
1-year foliage	24.59	29.41	29.47	29.17	(1.58)	26.06	(1.29)	0.119
Coarse roots	3.20	4.87	4.39	4.63	(0.48)	4.11	(0.39)	0.180
Fine roots	7.69	14.22	15.48	12.32	(1.42)	10.79	(1.16)	0.012

(SE)\*: standard error for main treatments, n=4.

(SE)#: standard error for seasonal treatments, n=6.

p : probability of treatment differences according to ANCOVA.

The data for the Control trees (Table 6.6) were converted to an areal basis using the compartment planting rate of 2250 stems/ha (Table 6.9). The nitrogen contents compare favourably with trees grown at Kaingaroa (Madgwick *et al.* 1977), and are considerably greater than the same aged trees on sand dunes at Woodhill Forest (Gadgil 1976, 1979). The level reported in Table 6.9 is not achieved at Woodhill until between age 4 and 5. This is not surprising, given that the Woodhill site is first rotation, and Bottle Lake third rotation. Slightly younger trees at Mount Gambiar (32 months) had above ground contents of 54.5 g N/tree (Fife and Nambiar 1982). These trees were growing on a fertile site and had also received 32-35 g N/tree. The contents are higher than the 49.3 g N/tree in the Controls in this study. Because of the closer spacing of the Mount Gambiar trees, the areal figure is much larger at 242 kg N/ha.

During the early years prior to canopy closure and a cycling of nitrogen through litterfall, there is a reliance on uptake from the soil. The net annual uptake of nitrogen into above ground components of radiata pine at Kaingaroa Forest between age 2 and 4 has been estimated to be 62 kg N/ha (Madgwick *et al.* 1977). A comparative figure was calculated for Bottle Lake by estimating the initial nitrogen content of Control trees in August 1983, at age 2 (Appendix 12). This was 11.7 g N/tree for above ground components only. The uptake from August 1983 to October 1984 was therefore 37.7 g N/tree. This is a slight

overestimate as an annual uptake figure, because the new season's growth had commenced by October. If half of the nitrogen in the current flush is assumed to have come from the soil rather than internal retranslocation, then the uptake between age 2 and 3 was 34.7 g N/tree, which is equivalent to 78 kg N/ha.

Table 6.9 Nitrogen content of unfertilized trees at Bottle Lake (Oct. 1984) at age 3.1 years.

Tree component	kg N/ha
Foliage	78
Twigs	20
Stem	13
Above ground	111
Below ground	29
TOTAL	140

Uptake of applied fertilizer may be calculated from Table 6.8 by using the difference method. The results of these estimates are given later in Section 6.3.5 where they are compared with the N-15 recoveries.

### 6.3.3 N-15 Uptake: Proportion of Nitrogen Derived From the Fertilizer.

Tables 6.10 and 6.11 show the percentage of nitrogen derived from the fertilizer (% Ndff) for the main and seasonal treatments, and the results from analyses of variance. The higher values in Table 6.10 are due to the higher rate of nitrogen applied (90 g v 30 g N). The similarity between the main treatments is striking (Table 6.10). The differences between tree components are small with the exception of 1-year foliage at >30% and fine roots at 17%. There are, however, some trends: both foliage and twigs have the highest value in 1-year material. Current material is intermediate in value and the older material lowest. There is a decrease from stem to coarse to fine roots.

For the seasonal treatments (Table 6.11) the patterns are similar except that the fine roots are not lower than other components. There is an interesting trend for foliage and, to a lesser extent, twigs between seasons of application. When the fertilizer was applied in autumn there was only older foliage as a sink, and the % Ndff was highest in this treatment. In summer the new foliage (1-year) was expanding and was a more important sink than the older foliage. This trend is statistically significant ( $p < 0.05$ ) between Autumn and Summer for current and older foliage.



Table 6.10 Proportion of nitrogen derived from the fertilizer in tree components at the end of the experiment (Oct. 1984) for the main treatments.

	Single	3-Split	9-Split	(SE)	P
	----- (%) Ndff -----				
Foliage					
current	27.7	25.6	25.9	(1.6)	0.608
1-year	30.4	32.0	30.4	(2.7)	0.888
older	21.3	24.7	21.9	(2.9)	0.689
Twigs					
current	23.2	26.0	26.7	(2.2)	0.526
1-year	26.1	27.0	27.0	(2.2)	0.940
older	22.4	25.0	24.7	(2.2)	0.669
Stem					
wood	23.2	23.6	25.5	(1.9)	0.683
bark	24.7	25.5	25.5	(2.3)	0.961
Roots					
coarse	22.1	22.5	23.5	(1.8)	0.844
fine	16.2	17.0	17.0	(2.4)	0.962

(SE): standard error.

p : probability of treatment differences according to ANOVA.

Table 6.11 Proportion of nitrogen derived from the fertilizer in tree components at the end of the experiment (Oct. 1984) for the seasonal treatments.

	Autumn	Spring	Summer	(SE)	P
	----- (%) Ndff -----				
Foliage					
current	7.8	9.2	12.5	(1.0)	0.096
1-year	10.4	12.8	14.3	(1.1)	0.190
older	13.1	8.9	7.2	(1.0)	0.054
Twigs					
current	8.0	9.2	12.4	(1.2)	0.169
1-year	9.2	10.3	11.7	(1.3)	0.516
older	9.5	9.6	9.4	(1.1)	0.988
Stem					
wood	8.7	8.8	9.4	(1.0)	0.863
bark	10.2	9.9	10.8	(1.3)	0.884
Roots					
coarse	9.0	9.3	9.5	(0.9)	0.936
fine	9.0	7.3	8.5	(1.0)	0.542

(SE): standard error.

P : probability of treatment differences according to ANOVA.

Heilman *et al.* (1982b) compared autumn and spring applications of urea in 7-year-old douglas fir. They found the Ndff to be higher in the older foliage on the autumn treatment in agreement with the trend reported here (Table 6.11). They suggested that this might be related to the better growth response on this treatment rather than an "available sink" hypothesis as offered in this study.

The greater % Ndff in the main treatments is not in proportion to the quantity of nitrogen applied, i.e. 3:1. This might be due to the larger biomass of main treatment trees rather than an indication of greater uptake in the seasonal treatments.

It is generally assumed that the greatest accumulation of labelled nitrogen occurs in the more metabolically active tree components (Knowles 1975, Mead and Pritchett 1975b, Worsnop 1978). The results in this study are in general concurrence with this view; foliage having the highest labelling, 1-year material higher than older, and bark higher than wood. There are, however, two noticeable exceptions: the current material and the fine roots are not the highest, even though they might be considered as the most active components at this early stage of the growing season. The nitrogen in the new flush probably comes from retranslocation and also soil nitrogen. If retranslocation from the 1-year foliage was the sole source, a similar enrichment of N-15 would be expected (c.f. autumn shoot data, Chapter 5). The lower labelling indicates dilution by a low enriched nitrogen source, such as from the soil or fine roots. Mead and Pritchett (1975b) also found the current flush to have a slightly lower % Ndff. They used this to support their contention that there was limited uptake of nitrogen from the fertilizer in the second growing season. In contrast, Nõmmik (1966), working with scots pine, found the highest labelling in the current needles even after three growing seasons. However, in a more recent study with scots pine, Melin *et al.* (1983) report the same foliar trends as in Tables 6.10 and 6.11.

The Ndff in fine roots for the main treatments is 17% (Table 6.10). This is compared with a peak of 30% in February (Figure 5.6b, Chapter 5). At this time the foliage was at 35-40% Ndff (Figure 5.4a, Chapter 5). Clearly the decline in fine root labelling has been much more dramatic than in the foliage. This decline is probably the result of dilution by lower enriched nitrogen. The pool of fertilizer derived ammonium was declining by February and the added N-15 may be assumed to be reaching equilibrium with the soil nitrogen (Chapter 5). Uptake from then onwards would be of soil nitrogen at a low enrichment, the fertilizer N-15 having been immobilized and become part of the large soil nitrogen pool. If the large fine root biomass was primarily formed after the major foliar flush (Chapter 3), then the considerable accumulation of nitrogen (Table 6.6) would be of a low enrichment and cause the relatively low figures in Table 6.10. For the seasonal treatment, where the response in fine root biomass was not so dramatic (Chapter 3) the Ndff fine roots is not markedly lower than other components (Table 6.11).

#### 6.3.4 N-15 Uptake - Tree Recovery

The total tree recovery for both rates of nitrogen (30 and 90 g N/tree) was about 20% (Table 6.12 and 6.13). The major sink was the 1-year foliage, where about 10% of the applied fertilizer was found. Fine roots contained <3%, and most other tree components <1% of that applied. Analyses of variance showed no significant differences between treatments.

Table 6.12 Fertilizer recovery in tree components at the end of the experiment (Oct. 1984) for the main treatments.

	Single	3-Split	9-Split	(SE)	P
	----- % of applied (90 g N) -----				
-----					
Foliage					
current	0.8	0.8	0.7	(0.12)	0.831
1-year	9.8	11.4	9.2	(0.98)	0.319
older	1.5	2.1	1.2	(0.29)	0.136
Twigs					
current	0.8	1.0	0.9	(0.10)	0.492
1-year	1.2	1.3	0.9	(0.10)	0.678
older	0.6	0.7	0.6	(0.10)	0.660
Stem					
1-year	0.3	0.5	0.4	(0.09)	0.395
lower wood	0.6	0.7	0.7	(0.08)	0.589
lower bark	0.5	0.6	0.6	(0.06)	0.720
-----	-----	-----	-----	-----	-----
Above ground	16.4	19.2	15.8	(1.56)	0.311
-----	-----	-----	-----	-----	-----
Roots					
stock	0.6	0.5	0.7	(0.07)	0.119
coarse	1.2	1.1	1.2	(0.17)	0.961
fine	2.6	2.9	2.1	(0.54)	0.407
-----	-----	-----	-----	-----	-----
Below ground	4.4	4.6	4.1	(0.54)	0.540
-----	-----	-----	-----	-----	-----
TOTAL	20.8	23.8	19.9	(1.99)	0.392

(SE): standard error.

p : probability of treatment differences according to ANOVA.

Table 6.13 Fertilizer recovery in tree components at the end of the experiment (Oct. 1984) for the seasonal treatments.

	Autumn	Spring	Summer	(SE)	P
	----- % of applied (30 g N) -----				
-----					
Foliage					
current	0.7	1.1	0.7	(0.24)	0.527
1-year	7.6	12.1	10.6	(1.68)	0.299
older	1.9	1.7	1.2	(0.26)	0.255
Twigs					
current	0.9	1.1	0.9	(0.11)	0.523
1-year	1.0	1.0	1.1	(0.03)	0.138
older	0.7	0.7	0.7	(0.15)	0.975
Stem					
1-year	0.3	0.4	0.2	(0.09)	0.290
lower wood	0.6	0.7	0.5	(0.04)	0.248
lower bark	0.6	0.5	0.4	(0.05)	0.239
-----	-----	-----	-----	-----	-----
Above ground	14.3	19.3	16.3	(2.09)	0.361
-----	-----	-----	-----	-----	-----
Roots					
stock	0.5	0.6	0.5	(0.10)	0.685
coarse	1.1	1.4	1.2	(0.24)	0.615
fine	2.3	2.9	3.3	(0.71)	0.677
-----	-----	-----	-----	-----	-----
Below ground	3.9	5.0	4.9	(0.97)	0.719
-----	-----	-----	-----	-----	-----
TOTAL	18.3	24.3	21.2	(2.86)	0.435

(SE): standard error.

p : probability of treatment differences according to ANOVA.

Initial tree size was considered to be one factor in determining uptake and resulting in the wide range of individual tree recoveries (15-32%, Appendix 3). There was a positive correlation between initial D<sup>2</sup>H and tree recovery ( $r=0.62$  significance,  $P=0.003$ ). Accordingly a co-variance analysis was run using initial D<sup>2</sup>H to remove the effect of variable initial tree sizes (Table 6.14). The seasonal treatments were pooled and included in this analysis.

The adjusted means became even more similar. The total tree recovery was within the range reported for other field studies (Table 6.2). In particular, the results agree with the often quoted figure of 20% (Mead and Gadgil 1978, Melin *et al.* 1983, Knowles 1975). It is clear that regardless of season, rate, or the splitting of the application, the tree recovery of fertilizer was the same.

Table 6.14 Fertilizer recovery in selected tree components, adjusted for initial tree size differences.

Tree component	Main treatments				Seasonal treatments (SE)#		P
	Single	3-Split	9-Split	(SE)* % of applied			
TOTAL	20.5	22.5	20.6	(1.7)	22.1	(1.4)	0.753
Above ground	16.1	18.2	16.4	(1.3)	17.4	(1.1)	0.669
Below ground	4.4	4.3	4.2	(0.5)	4.7	(0.4)	0.888
1-year foliage	9.6	10.7	9.6	(0.9)	10.6	(0.8)	0.753
Coarse roots	1.2	1.1	1.2	(0.2)	1.2	(0.1)	0.978
Fine roots	2.5	2.7	2.3	(0.4)	3.0	(0.3)	0.507

(SE)\*: standard error for main treatments (n=4).

(SE)#: standard error for seasonal treatments (n=6).

p : probability of treatment differences according to ANCOVA, with initial D<sup>2</sup>H.

Urea can apparently be applied as efficiently in autumn as in early summer, even though climatic conditions and tree growth patterns are very different. Higher temperatures and a rapidly expanding foliage biomass in December had no beneficial effect on uptake. This tends to contradict recommendations made for applying nitrogen during periods of rapid crown expansion (Mead and Gadgill 1978). However, Ballard (1984) agrees that season *per se* probably does not determine the effectiveness of urea, but that associated climatic conditions will affect losses.

Pritchett (1979) suggests that the season of application may have little influence on fertilizer effectiveness in moderate climates. Even in harsher climates, where applications timed to coincide with root growth are particularly important, it is generally agreed that the season of application has less influence on fertilizer effectiveness than might be expected (Viro 1970).

The same recovery at rates of 30 and 90 g N/tree (equivalent to 50 and 150 kg N/ha) is in agreement with the results of Mead and Pritchett (1975b) who used 56 and 224 kg N/ha applied to slash pine. Lower tree recoveries with increasing rate are usually only apparent at very high rates (Miller *et al.* 1976).

The trees were unable to utilise smaller, more frequent doses any more efficiently than a single application. The similarity between seasons suggests that for each split application approximately 20% is

utilised by the tree. Apparently there are one or more factors limiting the trees' ability to utilise a pool of added nitrogen, for example,

- rooting density of young pines;
- sink size for uptake, storage and utilisation;
- competition for ammonium from the microflora;
- leaching.

Discussions of the questions "Why only 20%?" and "Why Single equals Split?" is given in Chapter 8, following the presentation of the soil recovery data in Chapter 7.

#### 6.3.5 Comparison of Fertilizer Recovery by the Difference and N-15 Methods.

Most workers using the difference method to calculate fertilizer recovery report the problem in assuming that all the additional nitrogen is actually derived from the fertilizer (e.g. Nõmmik and Möller 1981). Some N-15 papers report a priming effect (see Chapter 5) and attempt to quantify it (e.g. Heilman *et al.* 1982a, Nambiar and Bowen 1986). However, separate estimates of total tree uptake based on the difference and N-15 methods are rarely reported. This may be due to the use of N-15 to overcome some of the difficulties associated with the difference method. For example Mead and Pritchett (1975b) found that total nitrogen contents were not significantly influenced by fertilizer. Melin *et al.* (1983) did not include a control treatment in their experiment. Some studies in agricultural systems have compared the two methods, often with a view to quantifying the priming effect (e.g. Westerman and Kurtz 1973).

The two methods have been compared in this study in order to ascertain their relative merits, to check some of the assumptions made in using N-15 (Hauck and Bremner 1976), and to serve as an insight into some of the nitrogen processes in the soil-plant system. Uptake by the difference method was calculated as the difference between nitrogen contents in fertilized and Control trees as a percentage of the nitrogen applied. The data in Table 6.8 were used and the results are presented in Table 6.15 along with the N-15 recoveries from Table 6.14. Standard errors for adjusted means and the difference between adjusted means were calculated according to Steel and Torrie (1981, p.416).

If the actual nitrogen contents (Table 6.7) had been used to calculate uptake by the difference method, there would have been a number of apparently negative values. The use of co-variance analysis to remove initial tree size differences has resulted in better estimates, although there is still one anomalous figure (Table 6.15). The difference method is also encumbered with large standard errors, particularly for the seasonal treatments. The advantage of using N-15 is clearly evident in its lower variability.

Table 6.15 Comparison of fertilizer uptake calculated by the N-15 and difference methods.

Tree component	Method	Single	3-Split	9-Split	(SE)*	Seasonal	(SE)#
		----- % of applied -----				-----	
TOTAL	DIFF	16.3	17.2	14.4	(5.3)	11.8	(13.2)
	N-15	20.5	22.5	20.6	(1.7)	22.1	(1.4)
Above ground	DIFF	6.6	7.3	6.5	(4.1)	(-1.4)	(9.9)
	N-15	16.1	18.2	16.4	(1.3)	17.4	(1.1)
Below ground	DIFF	9.7	9.9	7.8	(2.7)	13.1	(6.9)
	N-15	4.4	4.3	4.2	(0.5)	4.7	(0.4)
1-year foliage	DIFF	5.3	5.4	5.1	(2.6)	4.9	(6.6)
	N-15	9.6	10.7	9.6	(0.9)	10.6	(0.8)
Coarse roots	DIFF	1.8	1.3	1.6	(0.7)	3.0	(3.4)
	N-15	1.2	1.1	1.2	(0.2)	1.2	(0.1)
Fine roots	DIFF	7.2	8.6	5.1	(2.4)	10.3	(6.1)
	N-15	2.5	2.7	2.3	(0.4)	3.0	(0.3)

(SE)\*: standard error for main treatments n=4.

(SE)#: standard error for seasonal treatment n=6.

DIFF : % recovery according to the difference method.

N-15 : % recovery according to the N-15 method.

The main interest, however, in Table 6.15, is the difference in uptake estimates between the two methods. The N-15 method suggests a greater recovery of fertilizer in the total tree. This might be explained as fertilized trees not utilising the available native soil nitrogen or a depressed availability of native soil nitrogen on fertilized plots, called a negative priming effect (Hauck and Bremner 1976, Westerman and Tucker 1974). This trend is accentuated for the above ground components, e.g. 1-year foliage. This corroborates the observed pattern for individual needle nitrogen contents described in the previous chapter.

The opposite trend is noted below ground, particularly for the fine roots. On fertilized trees this component represents a large nitrogen pool, especially in relation to Controls (Table 6.6). Consequently, the difference method estimates a large recovery of applied fertilizer in fine roots. This is at variance with the N-15 estimate. If we assume the N-15 estimate is valid, then the additional 4 grams in fine roots of fertilized trees has come from the native soil nitrogen pool. This could be due to the fertilizer promoting the

mineralization of native soil nitrogen (a priming effect) by microbial means (Westerman and Kurtz 1973), or by a chemical action (Laura 1975). It could also be argued that the large fertilized trees utilised a larger, constant soil nitrogen pool more efficiently (Fried and Broeshart 1974) or that fertilized trees exploited a larger volume of soil (Nambiar and Bowen 1986). The priming effect explanation (by whichever means) seems unlikely because this is only evident below ground, and a possible transitory effect observed in the foliage soon dissipated (Chapter 5). The argument for fertilized tree roots exploring a greater volume of soil is plausible but tempered by two observations. Firstly the plot volume was identical for all treatments, and secondly a large quantity of roots were found encircling the plastic.

There is a further explanation, which takes into account the duration of an available N-15 pool in the soil and the seasonal growth of the tree. There is some evidence that fertilizer nitrogen was no longer readily available by March 1984 (Chapter 5), i.e. it had been drawn into the organic phase of the nitrogen turnover system. Consequently any uptake in mid to late 1984 would be of ammonium mineralized from a large soil organic nitrogen pool of which only a small portion is likely to be labelled. If fine root growth occurred later in the season, this could explain the lower N-15 enrichment (Section 6.3.3). Alternatively there may have been a substantial turnover of fine roots without a high degree of retranslocation into new fine roots. No estimates of fine root turnover were made. It should be noted that a large proportion of the fine roots have been suberized, and it is usually the finest roots that undergo a rapid turnover. It seems likely therefore that the bulk of the response to fertilizer in the fine roots occurred after the readily available N-15 pool had come to an equilibrium. This contention supports the view that major periods of growth in root and shoot, within the year, are episodic (see Chapter 3).

#### 6.3.6 Accuracy of Estimates

The total nitrogen analysis was shown to be both accurate and precise. Above ground biomass was determined by complete sampling. Provided that representative subsamples were taken, the estimates of nitrogen content should be accurate. The sampling of only half the plot for coarse and fine roots was deemed adequate as any slightly asymmetrical root systems would be expected to cancel out across a treatment.

The N-15 samples were only analysed in duplicate for the stem wood. The precision level (% difference relative to the mean) varied from 0.6-5% compared with 0.7-2.6% for total nitrogen. Care was taken to critically assess N-15 results in comparison with other samples, e.g. some trees had lower atom % values which were consistent across tree components. Results which appeared dubious were reanalysed.



The estimates of fertilizer recovery within the tree were considered to be accurate within the bounds of the sampling procedures. In this regard, two main factors are considered:

- (i) the use of single tree plots when in practice fertilizer is utilised by a stand of trees.
- (ii) the arbitrary definition of where roots interface with the soil.

Isolated single tree plots have usually been used in N-15 field studies (Paavilainen 1973, Bjorkman *et al.* 1967, Heilman *et al.* 1982b, Melin *et al.* 1983) to overcome the problem of accounting for uptake by surrounding trees (Mead and Pritchett 1975b), and the utilisation of a larger soil volume by trees responding to fertilizer (Nambiar and Bowen 1986). Recently, the advantages of more natural stand conditions have been achieved by using large (113 m<sup>2</sup>), root isolated plots containing several trees (Nömmik pers.comm.). It can be argued that single tree root isolated plots give an artificially low rooting density with implications for efficient use of fertilizer. Indeed, the multiple tree plots cited above have given some of the highest estimates of tree recovery (Table 6.2).

The problem of utilisation by surrounding trees is unlikely to be totally eliminated by lateral isolation of roots. Heilman *et al.* (1982b) used plots isolated to a depth of 45 cm and considered that some of the unaccounted for fertilizer may have been removed by roots of adjacent trees.

The dense mat of plot tree roots around the plastic has already been noted (Chapter 3). It was also evident that surrounding trees had formed a root mat on the "outside" of the plastic with fine roots intermingling with plot tree roots at the base of the plastic (Figure 3.2). Accordingly the 1-year foliage of trees within one metre of Single treatment plots was sampled at the end of the experiment, and analysed for N-15. The height and diameter at the base of these surrounding trees were measured to estimate biomass (Appendix 13). The Single treatment was chosen because leaching of fertilizer below the depth of the plastic was most evident in this treatment (Chapter 5).

Approximately 1.5% of the fertilizer applied to each Single treatment plot was utilised by surrounding trees (Appendix 14). Clearly the root isolation was quite effective and this small additional uptake should not be a factor confounding the preceeding treatment analyses.

The results have been presented on a per tree basis. This presents no problems above ground, but the definition of the tree's below ground components is more equivocal. All roots were collected within the limitations of field sampling and the sorting procedures (Chapter 3). No attempt was made to quantify mycorrhizal fungal biomass (c.f. Fogel and Hunt 1979). Mycorrhizal roots and mantles were included in the fine root sample. Fungal hyphae permeating the soil were assumed to be included in the soil sampling. It was possible that a

"rhizosphere pool of nitrogen" was not adequately sampled by these methods. Accordingly the "sieved sand" collected when separating coarse and fine roots (Figure 3.4) was analysed for N-15. The N-15 enrichment was slightly greater than for the 0-10 cm soil sample, and about half of that for fine roots. The fertilizer recovered within this sieved sand was negligible. The methods were concluded to be sufficient to sample the root/soil zone for fertilizer recovery.

The only error possible for above ground was the removal of significant amounts of fertilizer in the intensive foliage sampling scheme. The amount removed was negligible.

## CHAPTER 7

RECOVERY OF FERTILIZER WITHIN THE SOIL PROFILE  
AND IMPLICATIONS OF LOSSES

## 7.1 INTRODUCTION

In the previous chapter it was shown that about 20% of the applied fertilizer was in the trees. A minor amount was shown to have volatilized, and there was an indication of leaching, particularly on the Single treatment (Chapter 5). This chapter details the quantity of fertilizer that could be accounted for in the soil profile at the end of the experiment and discusses some of the implications.

The retention of fertilizer nitrogen within the soil system may not be so important for tree growth, if it is primarily of benefit to the tree, not to the site (Miller 1981). However, with the increased use of fertilizers in forests, there has been concern that leaching may reduce water quality (Tamm *et al.* 1974, Neary and Leonard 1978). The principal concerns are eutrophication of water courses and elevated levels of nitrate in drinking water (Sands 1984).

There is usually a large spatial variability in the soil nitrogen content (Keeney 1980). The amount of nitrogen fertilizer added is usually small in relation to the total pool. It is therefore difficult to estimate fertilizer recovery by the difference method (Nõmmik and Möller 1981). The use of N-15 therefore has been particularly useful in tracing soil nitrogen pools following fertilization (Jansson 1971). However, one drawback of this method is the uncertainty surrounding the extent of isotope exchange processes which may overestimate recovery in the soil (Melin *et al.* 1983).

## 7.2 METHODS

7.2.1 Field Sampling

At the end of the experiment, just prior to the tree harvest, the soil was sampled to a depth of 90 cm. An Idaho Sand Auger (i.d. 90 mm) was used to collect sequential cores from 0-10, 10-30, 30-50, 50-70, and 70-90 cm depth. Three of these horizons cover the depth to which the lysimeters were placed (20, 40 and 80 cm). On each plot six random cores were taken and bulked by depth.

A bulk density sampler with volume 114 cm<sup>3</sup> was used to collect samples from the horizons specified above. Two samples per plot were taken from the surface horizon. After the roots were excavated, a vertical profile was available to sample into (Plate 3.3), and at least one sample per plot was taken at 20, 40, 60 and 80 cm depths.

### 7.2.2 Laboratory

The bulk density samples were oven dried at 103° C and then weighed to the nearest milligram.

The bulked soil samples were air dried and subsequently split using a divider to give approximately 500 g samples. These were dry sieved (0.5 mm) to remove roots. For the 10-90 cm samples the organic matter retained on this sieve was ground with a pestle and mortar and returned to the sand for thorough mixing. For the 0-10 cm samples where most of the organic matter was, it was necessary to grind the samples in a ring and puck mill to ensure good mixing.

7.2.2.1 Nitrogen Analysis For the 0-10 cm samples, 0.5 g was digested and for the lower horizons, 2.0 g. The salicylic acid Kjeldahl modification was used (Bremner and Mulvaney 1982) and total N and N-15 determined as described in Section 5.2.4.2.

All samples were analysed at least in duplicate. The usual precision level was 3 %, i.e. the percentage difference between duplicates relative to their mean. Analyses were repeated if this value exceeded 5 %. A standard soil sample was obtained from Dr. L.G Greenfield and analysed with batches of soil samples. The agreement with his values was excellent.

7.2.2.2 Organic Matter Content Soil organic matter in each horizon was estimated, in duplicate, by loss on ignition at 430° C (Davies 1974). 5 g of ground and mixed sample were placed in a muffle furnace for 24 hours. Davies (1974) reported that this method gave very similar results to the wet oxidation method of Walkley and Black (1934). The precision level was 3%. The carbon content of soil was estimated from the organic matter content by using a conversion factor of 0.58 (Nicholson 1984).

### 7.2.3 Calculations

From the total-N, N-15, bulk density and plot volume data the recovery of applied nitrogen was calculated:

% recovery of applied nitrogen =

$$\frac{\pi r^2 h \times B.D. \times \%N \times 100 \times (c - b)}{(a - b)}$$

g N applied

where  $\pi r^2 h$  is the volume of the particular soil horizon, B.D. is bulk density, and a, b and c are atom % N-15 in the fertilizer,

unfertilized and fertilized samples respectively. Clearly there are many factors affecting the accuracy of this estimate, the appropriate volume of soil and bulk density being the two most critical (Khanna and Ulrich 1984).

### 7.3 RESULTS AND DISCUSSION

#### 7.3.1 Total Nitrogen Status of soil

The top 10 cm has the highest nitrogen concentrations, with the values then decreasing down the profile (Table 7.1). There was apparently a good relationship between nitrogen concentration and soil organic matter content (S.O.M.) in the 0-10 cm horizon (Table 7.2).

Table 7.1 Mean soil nitrogen concentration at the end of the experiment (Oct. 1984) for all plots.

Depth (cm)	% N air dry weight	(SE)	P
0-10	0.0637	(0.0065)	0.274
10-30	0.0191	(0.0009)	0.114
30-50	0.0113	(0.0004)	0.210
50-70	0.0083	(0.0003)	0.363
70-90	0.0067	(0.0001)	0.079

(SE): standard error.

p : probability of treatment differences according to ANOVA.

Table 7.2 Mean nitrogen concentration and soil organic matter in the 0-10 cm horizon at the end of the experiment (Oct. 1984) for all treatments.

Treatment	% N	% S.O.M.
Control	0.068	3.90
Single	0.086	4.65
3-Split	0.047	2.89
9-split	0.084	4.79
Autumn	0.040	2.41
Spring	0.041	2.66
Summer	0.053	3.81

The relationship is formalised in the following equation:

$$\%N = 0.0167 \text{ (S.D.M.)} + 0.0019 \quad r^2 = 0.90, \quad n = 22$$

This is similar to the relationship reported across several sites in South Australia (Nambiar and Cellier 1985).

$$\%N = 0.0149 \text{ (S.D.M.)} + 0.0080 \quad r^2 = 0.86 \quad n = 20$$

Such relationships are not unusual given that most nitrogen in forest soil is bound in the organic form (Pritchett 1979).

Hunter and Hoy (1983) report values of 0.01 - 0.02% N for the top 10 cm of mineral soil in a chlorotic stand of radiata pine growing on coastal sands. Gadgil *et al.* (1984), in a long-term trial at Woodhill Forest on coastal sand, report values for 0-10 cm and 60-70 cm. The upper horizon values range from 0.01-0.03% N and the lower fluctuated at about 0.01% N. There were no significant differences between control, lupin and fertilizer treatments. The upper horizon in this study has higher nitrogen concentrations, probably as a result of organic matter accumulation over two rotations. However, differences in soil sampling should also be considered.

On a site with a similar history of radiata pine crops in South Australia (Nambiar and Bowen 1986), the 0-10 cm nitrogen concentration was 0.03-0.04%. The Bottle Lake site would seem to have a higher nitrogen status than many of those studied on sand.

The nitrogen content of each plot was calculated using an average bulk density for each horizon (Table 7.3). The bulk density for 0-10 cm

Table 7.3 Mean soil nitrogen content by horizon at the end of the experiment (Oct. 1984) for all plots.

Depth (cm)	Nitrogen content ----- g/plot -----	(SE)	P
0-10*	540	(46)	0.199
0-10#	561	(57)	0.268
10-30	393	(18)	0.115
30-50	239	(8)	0.206
50-70	172	(5)	0.366
70-90	141	(3)	0.075
TOTAL*	1484	(71)	0.194

\* : calculated using individual plot bulk densities for 0-10 cm.

# : calculated using a pooled bulk density for all plots.

(SE): standard error.

p : probability of treatment differences according to ANOVA.

depth averaged  $1.24 \text{ g/cm}^3$  across all plots. The values for the lower horizons were 1.46, 1.49, 1.46 and  $1.48 \text{ g/cm}^3$  respectively. These are very similar to values given for a Mount Burr sand complex in South Australia (Nambiar and Bowen 1986). For 0-10 cm the results are also presented using individual plot bulk densities. This latter method is seen to reduce the variability and is preferred in subsequent calculations.

Generally there were no significant differences between treatments. As with nitrogen concentration, the organic matter content accounted for a large portion (93%) of the variation in nitrogen content. The lack of a fertilizer effect on total nitrogen precluded the use of the difference method to calculate recovery within the soil profile.

The total quantity of nitrogen in the plot (1484 g) is equivalent to 2106 kg N/ha. This compares favourably with the average figure in a study at Woodhill Forest of 1429 kg N/ha (Gadgil 1979).

### 7.3.2 Proportion of Soil-Nitrogen Derived from the Fertilizer

Tables 7.4 and 7.5 give the proportion of soil nitrogen derived from the fertilizer. Fertilizer was detected at all levels in the profile. There was significantly more labelled nitrogen in the split treatments than in the Single. There were no significant differences between seasonal treatments.

The lower values for the Single treatment are attributed to fertilizer moving below 90 cm as indicated by the leaching data (Chapter 5).

Table 7.4 Proportion of soil nitrogen derived from fertilizer at the end of the experiment (Oct. 1984); main treatments.

Depth (cm)	Single	3-Split	9-Split	(SE)	P	
	----- % Ndff -----				Single vs Split	3 vs 9 Split
0-10	2.8	6.9	5.4	(0.73)	0.005	0.204
10-30	1.7	3.7	4.1	(0.46)	0.004	0.602
30-50	1.6	2.0	2.7	(0.27)	0.049	0.094
50-70	0.9	1.2	1.4	(0.11)	0.040	0.199
70-90	0.7	0.6	1.1	(0.13)	0.514	0.035

(SE): standard error.

p : probability of differences between specified treatments according to single degree of freedom contrasts in ANOVA.

Table 7.5 Proportion of soil nitrogen derived from fertilizer at the end of the experiment; seasonal treatments.

Depth (cm)	Autumn	Spring	Summer	(SE)	P
	----- % Ndff -----				
0-10	2.6	2.7	3.1	(0.32)	0.626
10-30	1.3	2.2	1.3	(0.35)	0.258
30-50	0.4	0.7	0.8	(0.09)	0.118
50-70	0.6	0.4	0.8	(0.31)	0.692
70-90	0.1	0.3	0.3	(0.05)	0.113

(SE): standard error.

p : probability of treatment differences according to ANOVA.

### 7.3.3 Soil Fertilizer Recovery

From the soil nitrogen content and labelled nitrogen data in previous sections the recovery of applied fertilizer was calculated (Tables 7.6 and 7.7). Over half of that recovered was in the top 10 cm of soil and about 80% within 30 cm. Recovery diminished rapidly with depth.

Table 7.6 Recovery of applied fertilizer in the soil profile at the end of the experiment (Oct. 1984); main treatments.

Depth (cm)	Single	3-Split	9-Split	(SE)	----- P -----	
	----- % of applied (90 gN) -----				Single vs Split	3 vs 9 Split
0-10	20.9	32.1	37.8	(4.97)	0.046	0.441
10-30	9.6	13.5	17.6	(1.58)	0.013	0.099
30-50	4.9	5.0	7.7	(1.00)	0.253	0.084
50-70	2.0	2.2	2.6	(0.26)	0.235	0.341
70-90	1.2	1.0	1.7	(0.22)	0.712	0.054
TOTAL	38.6	53.9	67.5	(6.41)	0.021	0.168

(SE): standard error.

p : probability of differences between specified treatments according to single degrees of freedom contrasts in ANOVA.



Table 7.7 Recovery of fertilizer in the soil profile at the end of the experiment (Oct. 1984); seasonal treatments.

Depth (cm)	Autumn	Spring	Summer	(SE)	P
	----- % of applied (30 gN) -----			-----	
0-10	32.3	34.3	50.9	(9.73)	0.437
10-30	14.7	27.8	17.5	(3.59)	0.155
30-50	2.9	5.8	6.2	(1.27)	0.276
50-70	3.3	2.6	4.9	(1.40)	0.559
70-90	0.5	1.6	1.3	(0.28)	0.128
TOTAL	53.7	72.3	80.8	(13.43)	0.447

(SE): standard error.

p : probability of treatment differences according to ANOVA.

Significantly more fertilizer was recovered in the split treatments than the Single treatment. More fertilizer was recovered at all depths for the 9-Split treatment, although not significantly ( $p < 0.05$ ) above the 3-Split treatment. There were no significant differences between the seasonal treatments.

The greater recovery at the lower application rate (50 v 150 kg N/ha) agrees with other studies (e.g. Mead and Pritchett 1975b, Nõmmik and Möller 1981). Tree uptake was similar between rates (Chapter 6), and volatilization of ammonia was negligible (Chapter 5). The greater loss from the higher application rate may be attributed primarily to leaching losses. The leaching data in Chapter 5 supports this contention. Greater leaching with increasing application rate was also shown by Overrein (1969) in lysimeter studies with tracer nitrogen. The other possible loss mechanism, which was not measured, is denitrification. The production of dinitrogen and nitrous oxides requires nitrate as an intermediary, and is often mediated by bacteria under anaerobic conditions (Keeney 1980). Nitrate was present (Chapter 5) but anaerobic conditions were unlikely.

Leaching is a complex process with a variety of chemical and biological factors regulating its importance. If the ammonium resulting from urea hydrolysis is not held within the soil or taken up by tree roots, it will be prone to leaching through the profile. The ammonium ion may be absorbed onto the cation exchange sites, be fixed chemically onto organic matter or utilised by the soil heterotrophs. The ability of the soil system to absorb ammonium is seen to be related to the quantity of nitrogen applied. With a single application of 90 g N the system became saturated and significant leaching occurred, e.g. the difference between total soil recovery for Single and split treatments was 20 g N. The soil organic matter contents might be related to the recovery of fertilizer. Whether microbial immobilization or chemical

fixation of ammonium predominated the processes would be expected to be linked with soil organic matter. The contention is further supported by the knowledge that urea recoveries are high in stands with substantial litter layers (Popovic and Nõmmik 1972).

Accordingly, the relationship between fertilizer recovery and soil organic matter for the 0-10 cm horizon on all plots was calculated (Table 7.8). The non-significance for all plots combined may be due to another factor masking any relationship. This could be excessive leaching on the Single treatment and so individual treatment regressions were also run (Table 7.8). There was no significant relationship for the Single treatment. This might indicate that leaching was occurring before immobilization was possible. On the other treatments where substantially less leaching occurs there is generally a positive relationship indicating the importance of organic matter in retaining fertilizer within this upper horizon.

Table 7.8 Relationships between recovery of fertilizer and soil organic matter in the 0-10 cm horizon. Regressions are of the form: soil recovery % = a + b (S.O.M.) %.

Treatment	a	b	r <sup>2</sup>	P	n
All	24.0	2.3	0.11	0.186	18
Single	2.3	4.0	0.25	0.502	4
3-Split	-0.5	11.2	0.99	0.007	4
9-Split	28.8	1.9	0.57	0.244	4
Seasonal	0.7	13.0	0.93	0.002	6
3+9+Seasonal	24.0	3.7	0.36	0.024	14

a,b: regression co-efficients.

r<sup>2</sup>: proportion of variation accounted for by regression.

p : significance of regression.

n : number of plots.

If the immobilization process is primarily microbial, then substrate conditions must be considered. Rather than organic matter *per se* the carbon to nitrogen (C:N) ratio may be a better index of the likely immobilization of fertilizer. High C:N ratios are likely to indicate that nitrogen is limiting microbial growth. Accordingly there may have been greater immobilization on plots with high C:N ratios. The analysis in Table 7.8 was repeated using C:N ratios for the 0-10 cm soil horizon as the dependent variable (Table 7.9). The C:N ratio refers to the plots at the end of the experiment. Carbon concentration was assumed to equal 0.58 x organic matter. The relationship was slightly better although the Single treatment remained non-significant.

The analysis should be treated with caution as the majority of plots had C:N ratios about 34:1. There was one outlier (Tree 859,

Summer treatment) with a C:N ratio of 50:1, which was the primary cause of the positive relationship. Both organic matter and C:N ratio relationships were also tested for fertilizer recovery at 10-30 cm (data not presented). The trends were similar and again tree 859 was an outlier with a C:N ratio of 98:1.

Table 7.9 Relationships between recovery of fertilizer and soil C:N ratio in the 0-10 cm horizon: soil recovery % =  $a + b$  (C:N)

Treatment	a	b	r <sup>2</sup>	P	n
All	-16.4	1.41	0.31	0.016	18
3+9+Seasonal	-14.3	1.42	0.40	0.015	14

a, b, r<sup>2</sup>, P and n as for Table 7.8.

There was, therefore, some correlation between fertilizer recovery and the soil carbon status, but a more useful index to use would probably be some measure of available carbon and available nitrogen (Johnson *et al.* 1980).

#### 7.3.4 Amount of Nitrogen Leached

The loss to leaching can be calculated if this is assumed to equal the portion unaccounted for in the tree and soil. Accordingly about 36, 20 and 10 grams of nitrogen from the Single, 3-Split and 9-Split treatments respectively were leached below 90 cm depth. A small proportion of this was utilised by surrounding trees (Chapter 6), but the majority was probably lost to trees. Sands (1984) cites unpublished data of Nambiar showing the proportion of 84 g N (as ammonium sulphate) that leached from 2-year-old radiata pine on a weed free, sandy site in South Australia. By 116 days after fertilization, 62% had leached below 32 cm. In this study about 49% had leached below 30 cm after 410 days, on the Single treatment. The difference may be due to the method of application. In South Australia fertilizer is broadcast in 45 cm wide bands at this age (Woods 1976).

#### 7.3.5 Importance of Leaching

The loss of fertilizer from the system is obviously undesirable from an economic and environmental viewpoint. Although most roots occur within the top 50 cm of soil, it is possible that nitrogen leached to depth is still available to trees. For example the limited uptake of fertilizer by surrounding trees must have been by roots at, or below 75 cm. The plastic surround could however have induced roots to grow deeper than would be the case in an unrestricted soil volume. Two-year-old radiata pine is reported to be able to absorb water from

at least 2 m depth on sand (Sands and Nambiar 1984). This may suggest that nitrogen lost below the sampled horizon in this study could still be utilised by trees.

The higher loss of fertilizer on the Single treatment plots, i.e. about 20 g N, implies a reduced long-term effectiveness of applied nitrogen for this treatment. However, this additional nitrogen retained following split applications represents only about 1.3% of the total nitrogen pool. Unless this is in a readily available form such as short term immobilization in the microflora it is unlikely to be of great benefit to the tree in the longer term.

Whilst the leaching of nitrogen *per se* may not be detrimental to tree growth, the associated chemical effects must be considered. Urea fertilization has been shown to promote the leaching of cations in laboratory studies (Ballard 1979, Allen 1981) and in the field (Pang and McCullough 1982). The mechanism of this leaching is the displacement of cations from exchange sites by fertilizer ammonium, and subsequent transport to depth by anions. The carbonate ion, a product of hydrolysis, and nitrate as a result of nitrification are critical in this respect. The latter was known to be present (Chapter 5) and the former assumed to be, at least initially.

No analyses for cations in the soil solution were made. However, where greater leaching took place on the Single treatment, the net effect may be apparent in the nutrient content of trees. This was investigated by analysing the 1-year foliage (the main nutrient pool) at the end of the experiment for potassium, calcium and magnesium. An X-ray fluorescent spectrophotometer was used (Chapter 4). The concentration of these nutrients on the Single treatment was lower than the other treatments (Table 7.10). Single degree of freedom contrasts

Table 7.10 Foliar nutrient concentrations of 3.1-year-old radiata pine as affected by urea fertilizer after one growing season (Oct. 1984).

Nutrient	Control	Single	3-Split	9-Split	(SE)	P
----- % oven dry weight -----						
K	0.79	0.60	0.83	0.81	(0.062)	0.082
Ca	0.41	0.33	0.32	0.39	(0.025)	0.067
Mg	0.12	0.10	0.12	0.12	(0.010)	0.519

(SE): standard error.

p : probability of treatment differences according to ANOVA.

showed this effect to be highly significant for potassium. The values in Table 7.10 are obviously affected by such factors as initial

nutrient status and biomass as well as possible fertilizer induced changes in cation availability. However, the initial foliar nutrient concentrations were very similar (Table 4.9). The final biomass of fertilized trees was also similar (Chapter 3), thus vitiating a dilution hypothesis.

The actual nutrient contents of the 1-year foliage were calculated from Table 7.10 and the biomass figures (Chapter 3). These were analysed in an analysis of co-variance using initial D<sup>2</sup>H as the co-variate to remove variable tree size, and presumably nutrient content, effects. There was significantly less potassium in the Single treatment compared with the split treatments (Table 7.11). The higher potassium content in the 3-Split treatment reflects the pretreatment levels (Table 4.9). The potassium values were probably overestimated by about 10% (Appendix 8). This, however, does not affect treatment comparisons.

As all trees fertilized with 90 g N had a similar biomass, there is evidence for the leaching of potassium induced by the Single application of 90 g N. This was sufficient to restrict the uptake of potassium in comparison with trees fertilized by split applications. In practice this effect may be insignificant. The level of potassium in this foliage during February/March for the Single treatment was satisfactory at 0.9% (Chapter 4). The beneficial effects of split applications in reducing leaching and hence cation loss might be more important on less fertile sites.

Table 7.11 Nutrient content of 1-year foliage at the end of the experiment (Oct. 1984), adjusted for initial tree size.

Nutrient	Control	Single	3-Split	9-Split	(SE)	P
			(g)			
K	10.27	12.08	17.54	14.21	(1.006)	0.010
Ca	6.53	6.60	6.51	7.21	(0.526)	0.776
Mg	1.85	2.09	2.66	2.14	(0.197)	0.085

(SE): standard error.

p : probability of treatment differences according to ANOVA.

#### 7.3.6 Accuracy of Estimates

The sampling of forest soil to estimate the size of nitrogen pools is fraught with difficulty. Even the use of N-15 relies on assuming an evenly distributed labelling across the plot. The presence of old stumps and slash (not visible initially) and old rooting channels on some plots may have become preferential sites for

fertilizer accumulation.

The use of only 3 small cores per plot (Chapter 5) was shown to give highly variable total nitrogen concentrations. The use of six larger cores per plot and four plots per main treatment has hopefully overcome this variability. The situation with the lower replication on the seasonal treatments is more equivocal. For example, it is suggested that the soil recovery on the summer treatment is overestimated (Table 7.7) because plot 859 recorded a recovery of 103% excluding the tree (Appendix 3).

It is interesting to note that the grand mean for the 0-10 cm data using the Hoffer soil tubes was 0.084% N (Appendix 11). The comparative figure using the Idaho Sand Auger (above) was 0.086% N. The values for the 10-30 cm samples were 0.018 and 0.024% N for the Hoffer and Idaho samplers respectively. This discrepancy may be due to the possibility of some mixing of horizons using the Idaho Sand Auger.

There was some concern as to the correct plot radius to use in the calculations. The actual radius to the plastic was 1.5 m, but an unfertilized margin was left when applying fertilizer (Chapter 2). It is probable that in moving through the profile there was a lateral movement of nitrogen, so the use of a 1.5 m radius is assumed to be valid for 10-90 cm depths. The extent of lateral movement of fertilizer within the top 10 cm was unknown. If, for example, a plot radius of only 1.4 m was used, the estimated recovery of fertilizer (Tables 7.6 and 7.7) would be reduced by 14.8%.

## CHAPTER 8

## SYNTHESIS

## 8.1 RESUME OF EARLIER CHAPTERS

The aim of this study, as outlined in Chapters 1 and 2, was to investigate a possible method for improving the uptake of nitrogen fertilizer and retention within the ecosystem. A conventional Single application of urea at 150 kg N/ha (90 g N/tree) was compared with equal 3-Split and 9-Split applications applied from May to December. A Control was included as well as three seasonal treatments at 50 kg N/ha (30 g N/tree). Tree growth and initial distribution of fertilizer were monitored for 17 months. The experiment was then destructively sampled to ascertain biomass and distribution of fertilizer nitrogen within the ecosystem.

There was a 9% diameter response to 90 g N per tree which occurred whether or not split applications were used. There was no detectable diameter response to 30 g N. There was no height response which was expected, given that the site is not severely deficient in nitrogen (foliar N = 1.47%).

Foliage analysis showed that total nitrogen content of needles increased through the year (Chapter 4). The uptake of fertilizer nitrogen was initially rapid regardless of treatments, but apparently not in proportion to the amount applied (Chapter 5). However, with time uptake of N-15 from the lower rates levelled off, whereas it continued to increase at the higher rate. According to foliar analysis, net uptake of N-15 ceased in March, some 6 months after the Single application, and 3 months after the final Split application. About this time, ammonium concentration in the soil solution on fertilized plots returned to Control levels.

Uptake of nitrogen in fertilized trees after March was assumed to be from a "general" soil nitrogen pool which was only slightly enriched with residual N-15. The declining proportion of foliar nitrogen derived from the fertilizer later in the season confirmed this, as did some limited analyses of fine root samples. Soil samples from the Single treatment indicated that the total N-15 in the top 30 cm had stabilised at about 30% of that applied, within four months of application. It was concluded that the major losses from this high rate of nitrogen occurred by leaching of both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ .

The foliar N-15 data (Chapter 5) suggested that there was no effect of split applications on uptake of fertilizer. This was confirmed after the final harvest. Irrespective of Single or Split applications, there was a 20% recovery of applied fertilizer within the tree, i.e. about 18 g N. This resulted in a 30% biomass response, with the greatest increase being in fine roots (80% over the Control) and

foliage and branches formed since fertilization (25%). This response pattern was attributed to limits on response above ground, with initial tree size being one factor. There was no such limit below ground where the response was viewed as an opportunistic adaption to colonise the soil in a young plantation.

The tree recovery of fertilizer in the seasonal treatments was also about 20%, i.e. 6 g N, and there was no indication of any advantage in applying fertilizer in either spring, summer or autumn. The amount of nitrogen taken up was insufficient to produce a detectable above ground response. There was, however, a significant below ground response.

At the final harvest the soil profile was sampled to determine fertilizer recovery to a depth of 90 cm (Chapter 7). There was significantly greater recovery on the split treatments (61% of that applied) than on the Single treatment (39%). The soil recovery for the seasonal treatments (69%) was similar to the split treatments, but significantly higher than the Single treatment. Clearly, the capacity for retention of ammonium on cation exchange sites and utilisation by the soil heterotrophs has been exceeded with the Single application of 90 g N.

## 8.2 TOTAL RECOVERY

The tree and soil recovery data from Chapters 6 and 7 have been combined (Table 8.1). Volatilization losses and uptake by surrounding trees probably add a further 1-5%. The recoveries are within the range reported from other N-15 studies (Table 8.2), although the Single application is quite low.

Table 8.1 Total recovery of fertilizer within the ecosystem.

Treatment	grams N applied	Recovery			
		Tree	Soil	Total	Unaccounted*
		----- % -----			
Single	90	20.8	38.6	59.4	40.6
3-Split	90	23.8	53.9	77.7	22.3
9-Split	90	19.9	67.5	87.4	12.6
Autumn	30	18.3	53.7	72.0	28.0
Spring	30	24.3	72.3	96.6	3.4
Summer	30	21.2	80.8	102.0	-

\*: includes 1-5% volatilized as ammonia or utilised by surrounding trees.



Table 8.2 Recovery of applied nitrogen in forest ecosystems - N-15 studies.\*

Fertilizer	Rate kg N/ha	Biomass	Recovery		Reference#
			Soil and	Total	
			litter		
			----- % -----		
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	60	20.8	58.5	79.3	Bjorkmen <i>et al.</i> 1967
CO(NH <sub>2</sub> ) <sub>2</sub>	53	21.7	44.0	65.7	Paavilainen 1973
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	56	24.7	29.6	54.2	Mead and Pritchett
	224	26.6	17.9	44.5	1975b
CO(NH <sub>2</sub> ) <sub>2</sub>	224	30	38	68	Heilman <i>et al.</i> 1982b
NH <sub>4</sub> NO <sub>3</sub>	100	30	52	82	Melin <i>et al.</i> 1983
CO(NH <sub>2</sub> ) <sub>2</sub>	150	21	66	87	Nömmik pers. comm.
NH <sub>4</sub> NO <sub>3</sub>	150	34	54	88	Nömmik pers. comm.
NH <sub>4</sub> NO <sub>3</sub>	50	30	70	100	Nömmik pers. comm.

\*: see also Knowles (1975, Table 5). and Ballard (1980, Table 3).

#: see Table 6.2 for stand details.

Analyses of variance showed significantly different total recoveries between Single and split applications, and between the Single and seasonal treatments (Table 8.3). Given that tree uptake was the same, these differences are due entirely to the greater losses from the Single application.

Table 8.3 Probability (p) of treatment differences in total recovery according to ANOVA and single degree of freedom contrasts.

Comparison	P
Between main treatments	0.009
Single vs. Split	0.004
Between 3 and 9-Split	0.196
Between seasonal treatments	0.320
Single vs. seasonal treatments (pooled)	0.025

### 8.3 DISCUSSION

It is clear that one objective of splitting the application has been met, i.e. a greater retention of fertilizer within the ecosystem. Indeed, the recovery in the 9-Split treatment is better than in a continuous fertilizer and irrigation treatment with scots pine in Sweden where 76% of applied nitrogen was accounted for (Ingestad *et al.*

1981). Whether this increased recovery could lead to a greater nutrient flux density and long-term site improvement as argued by Ingestad and Agren (1984) is debatable. In this study the amount of fertilizer retained within the soil was less than 2% of the total nitrogen pool. It is probable that there will be no long-term improvement and thus it is the amount of fertilizer that the tree can initially use that is critical (Miller 1981).

The other proposed benefit from split applications, i.e. increased tree uptake, has not eventuated. Indeed, the similarity between treatments was striking. The study was designed to maximise tree uptake of nitrogen by applying it to young pines during an exponential phase of growth and by controlling the competing vegetation. The application rate was conventional for commercial forestry operations and was not excessive given the tree's requirements for nitrogen at this age (Chapter 6). Despite these factors the tree recovery was similar to that reported from a variety of studies (Table 6.2).

The reasons for a uniform recovery across treatments must be that uptake is limited by tree characteristics and/or processes that regulate nitrogen availability within the soil.

### 8.3.1 Tree Characteristics

A positive correlation was found between initial D<sup>2</sup>H and tree uptake (Chapter 6). Similarly, there was a positive correlation between the major sink for applied fertilizer (older foliage) and tree uptake. This latter variable explained 55% of the variation in uptake. This effect of sink size may also be illustrated by comparing data from the Single and seasonal treatments with a recent study on young radiata pine in South Australia (Table 8.4).

Table 8.4 Effect of tree size on recovery of applied nitrogen in young radiata pine on sandy soils.

Age (months)	Application (g N/tree)	Height (m)	Biomass (g/tree)	Uptake (%)	Reference
2	4*	0.3	5	6.4	Nambiar and Bowen 1986
13	18*	0.8	142	17.7	
24	30	1.6	900#	21.3	This study
24	90	-	-	20.8	

\*: ammonium sulphate.

#: from Appendix 4.

The comparison must be tentative given different fertilizers and sites. The increasing uptake with age and tree size may also be related to rooting density. Nambiar (1983) reports a rooting density of 0.05-0.1 cm/cm<sup>3</sup> in 15-month-old radiata pine, which has more than doubled by age 34 months, but had clearly not increased in accordance with biomass. The increased uptake, indicated in Table 8.4, from 13-24 months is not large and suggests a further limiting factor. An asymptote is to be expected as mature trees do not show especially high recoveries.

The tree's initial nutritional status might have influenced the uptake of fertilizer nitrogen. On some trees both sulphur and magnesium apparently approached marginal levels during the experiment. However, there was no evidence that either element limited nitrogen uptake (Chapter 4) or growth response (Chapter 3). There were significantly lower foliar potassium concentrations on Single treatment trees, apparently as a result of leaching of K<sup>+</sup> in conjunction with NO<sub>3</sub><sup>-</sup> or HCO<sub>3</sub><sup>-</sup>, resulting from urea application. However, potassium levels remained satisfactory in the foliage at all times.

There were appreciable amounts of nitrate in the soil solution on all plots. This was during a period of high demand for nitrogen within the foliage and a declining ammonium content in the soil solution. If the trees were not efficient at utilising nitrate, then a decreased uptake of fertilizer would be expected on this nitrifying site. Utilisation of nitrate is dependent upon nitrate reductase activity (NRA) in the roots, which is induced by the ion's presence (Larcher 1983). Adams and Attiwill (1983) induced higher levels of NRA in root tissues following applications of 1000 kg N/ha to radiata pine. At 500 kg N/ha the increased NRA was not significant. At the lower rates of application in this study, there may have been little or no increase in NRA with a possible correspondingly low utilisation of nitrate.

#### 8.2.2 Leaching

If denitrification is assumed to be negligible (see p.135) then the majority of the unaccounted for fertilizer (Table 8.1) can be attributed to leaching losses. There was an initial movement of fertilizer down the profile after application. The extent of this was partly dependent on rainfall (Table 2.4). High concentrations of ammonium occurred at greater soil depths for longer periods following the Spring and Summer applications, when appreciable rain fell. However, there were no significant differences between seasonal treatments for tree or soil recovery and the quantities leached were apparently small (Table 8.1). Leaching was, therefore, not considered to be an important mechanism regulating the availability of nitrogen to trees at an application of 30 g N/tree.

Leaching was similarly not a major loss for the split applications. There was, however, a major loss of nitrogen by leaching

on the Single treatment. It would be interesting to know whether the extra 20 g N lost in comparison with split applications would have been available for tree uptake, if leaching had not occurred. It seems likely that some would be, but the actual amount would depend on the extent of immobilization.

### 8.3.3 Immobilization

The other major sink for applied N-15 is immobilization. In the seasonal treatments the foliar N-15 content stabilises, about 160 days after fertilization. As leaching was not considered to be a major loss, this was probably due to a gradual immobilization of N-15 by the soil heterotrophs.

On the main treatments the same asymptote for foliar N-15 content was reached. If foliar N-15 analysis is a true indication of N-15 availability in the soil, then an equilibrium with native soil nitrogen was reached in March, for Single and Split treatments. Processes are apparently acting on three differently sized pools, i.e. 90 g N applied in August and one of 30 and 10 g N applied in December to bring about equilibrium conditions at the same time. Both leaching and immobilization will bring this about. The greater leaching on the Single treatment will have the effect of evening up the size of N-15 pool available for uptake and immobilization. The rate of immobilization may also differ between treatments. Foster *et al.* (1985b) report that increasing immobilization of urea nitrogen up to 200 kg N/ha is due to increased microbial activity.

Johnson *et al.* (1980) consider that trees are at a great disadvantage to soil micro-organisms in terms of taking advantage of the increased ammonium availability following fertilization. This is apparent in this study if the high recoveries in the soil are mainly microbially mediated. However, what is the nature of this competition? Is it a direct effect with soil micro-organisms utilizing nitrogen more efficiently than roots? Or is it a consequence of the spatial distribution of roots and their competitors on the plot? The latter are presumably ubiquitous across the plot and will come into contact with the evenly broadcast application (Figure 2.3). The roots of 2-year-old trees may be visualised as occupying certain narrow sectors of the plot. Thus low rooting density may be reducing the efficient use of fertilizer. Utilisation of fertilizer by the tree between these sectors must rely on at least three factors:

- root extension;
- diffusion to the root sector;
- mycorrhizal hyphae permeating between sectors.

The extent to which the trees absorbing capacity increased over the time frame of available N-15 was unknown. The Split applications could have "primed" the tree, by increasing root growth for example,

thus enabling a greater utilisation of subsequent applications. Root growth probably did occur during the period of fertilizer applications, but increased uptake has not eventuated. Again the tree cannot be studied in isolation. Previous applications are also likely to have affected the soil environment, with elevated pH and ammonium levels along with increased microbial activity. Thus the tree may theoretically be able to utilize more nitrogen, but the soil heterotrophs also have the capacity to immobilize greater quantities.

#### 8.3.4 Summary

Rooting density, sink size, heterotroph competition and leaching have probably all acted to restrict the uptake of applied nitrogen to an average of 20%. The actual range of 15-32% can be partially accounted for by initial tree size.

The efficiency of Single and Split applications appears to depend upon the relative importance of leaching and immobilization. In this study there were no immediate benefits to the tree in splitting the application. Indeed, in the absence of leaching, a Single application could be advantageous, because an available nitrogen pool might last longer. It is probable that the efficiency of split applications is hindered by soil immobilization processes which may be greater for later applications. For a Single application the extent of leaching is apparently the more critical process in limiting the efficient use of fertilizer.

#### 8.4 SUGGESTIONS FOR FUTURE RESEARCH

In this study the tree was studied in greatest detail. Only the net effects of leaching and immobilization were quantified, and no data were presented for actual rooting density. Whilst the possible benefits of split applications for tree uptake have been disproven, the actual mechanisms were not fully elucidated. In particular the processes leading to the reduction in available nitrogen from fertilizer need further study. This should show whether smaller, more frequent doses are primarily benefiting the soil microflora, rather than the tree. The root environment requires further laborious study. For example, what is the spatial distribution of roots? And is it possible to place fertilizer where competition by soil organisms is less?

There would be some merit in conducting certain tests under controlled conditions to study one or two processes in detail. However, as has been indicated, a number of factors operate to determine fertilizer uptake efficiency and an integrated approach to determine the net effects, as studied here, also has benefits. One drawback of a field study of this type is accepting the environmental conditions prevalent at the time. This was overcome for volatilization, but not for leaching. A particularly useful study would be to repeat the experiment where leaching is minimised. In a pot study with young

douglas fir, Pang (1984) reported a 40% tree recovery from an equivalent of 200 kg N/ha, which is the highest value from any known N-15 studies with trees.

A simple model to predict the amount of fertilizer utilised by the trees could be constructed. Three major factors have been discussed earlier: initial tree size, immobilization, and leaching. The first of these was used to account for some of the variability in uptake. The use of soil organic matter content, as a variable related to immobilization, did not significantly improve the amount of variability accounted for. There were no independent estimates of leaching available for incorporation in a model. Further studies need to quantify factors which account for immobilization and leaching.

Foliar nitrogen analysis was used in this study to monitor the trees's nitrogen status. The method is useful, but requires care in interpretation, and a continuing research is needed to understand what a particular value actually means. For example, how does one compare the 1.1% N concentration in a more productive young radiata pine in South Australia (Fife and Nambiar 1982) with the 1.5% N at Bottle Lake?

Studies with nitrogen fertilizer should always include at least an appraisal of the status of other nutrients. A number of results from this study need more attention: the S:N interaction, the possible  $Mg^{++}:NH_4^+$  interaction, and the leaching of cations.

The major response below ground in this study needs to be assessed in a longer term study. Will this confer subsequent benefits on the trees' ability to absorb water and nutrients? In particular, would a repeat fertilizer application in the following year be more efficiently utilised? The possibility exists for obtaining a root response in the first year (perhaps with a low rate of nitrogen to minimize leaching, e.g. 30 g N/tree) which would enable larger applications in subsequence to be more efficiently utilised.

## 8.5 IMPLICATIONS FOR MANAGEMENT

The starting point for this study was the apparently low recovery of nitrogen fertilizer in the target organism: the tree. As fertilization is an investment in the crop, this low efficiency should be of concern to managers. As guardians of the environment, foresters also need to be aware of the often low recovery of fertilizer within the ecosystem.

If leaching is a problem on a particular site and retention within the soil system is important, then split applications are one solution available to the manager. Split applications of nitrogen may also have benefits over single applications, if the status of other nutrients is marginal.

The use of split applications appears to confer no immediate benefit to the tree. Any residual benefits from increased retention of nitrogen within the soil will depend upon the amount in relation to the nitrogen capital of the site and whether it is in a more available

form.

This study indicates that fertilizer can be applied with equal efficiency in different seasons. However, with applications of urea pellets the possibility of volatilization losses at low moisture and high temperature would have to be considered by the forest manager. The most efficient way to apply nitrogen fertilizer appears to be as a single application, particularly if leaching losses are likely to be low.

This study suggests that it is possible to achieve a root response in young pines with a rate of nitrogen that is not susceptible to leaching. An application in the following year, when a greater rooting mass and sink size exist, may be more efficiently utilised.

*But, those attain'd, we tremble to survey  
The growing labours of the lengthen'd way,  
Th' increasing prospects tires our wand'ring eyes,  
Hills peep o'er hills, and Alps on Alps arise!*

Pope (1711)

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## APPENDICES

## Appendix 1

Fine Root Estimation

Four root systems were washed out and there was concern about the loss of fine roots. This was confirmed by the biomass data (Table A.1).

Table A.1 Fine root biomass for the main treatments.

Control	Single	3-Split	9-Split
grams			
(413)	(501)	(559)	(304) washed out
804	1244	1504	1435
711	1309	1605	1601
700	793	1370	769

These low values were inappropriate for the biomass and subsequent nitrogen recovery calculations. Accordingly estimates of the fine root biomass for these four trees were made.

There were data available for 18 trees to develop estimating equations (3 Controls, 6 Seasonal and 9 Main treatments trees). However, the pooling of data across treatments is unwise, given the apparent magnitude of response. The relationship between above and below ground biomass was tested on a treatment basis. For the three "correct" Control trees the following linear equation described the relationship:

$$\text{Below ground (wt.)} = -948 + 0.591 (\text{Above ground wt.}) \quad r^2 = 0.894$$

It is, however, meaningless to have a non zero root weight when shoot weight is zero. The regression intercept may thus be set at zero (c.f. Carlson and Presig 1980) to give the equation:

$$\text{Below ground (wt.)} = 0.435 (\text{Above ground}) \quad r^2 = 0.996, \text{ SE} = 0.00167$$

This estimates a below ground biomass for the washed out Control tree of 2394 g. Subtraction of the rootstock and coarse root weight leaves an estimate of 741 g fine root. This value is used in subsequent calculation.

There was no correlation between above and below ground biomass for the main treatments. Accordingly the correlation between fine root biomass and other tree components was investigated. The best

correlation was with 1-year foliage. The equation below incorporates the seasonal treatment trees as well.

$$\begin{aligned}\text{Fine root wt.} &= -237 + 0.771 \text{ (1-year foliage wt.)} \\ r^2 &= 0.518 \\ P &= 0.0015 \\ SE &= 336 \text{ and } 0.193\end{aligned}$$

This equation estimates fine root biomasses of 1492, 1873 and 1365 g for the washed out trees from the Single, 3-Split and 9-Split treatments respectively.

#### Comments

The equation relating fine root biomass to 1-year foliage for the three Control trees only, is nonsignificant. However, the estimate obtained if it is used for the washed out tree is 738 g, the same as the value estimated above.

Jackson and Chittenden (1981) published an equation for estimating fine roots (< 2 mm) from foliage biomass:

$$\text{Fine root wt.} = -121.35 + 0.526 \text{ (Foliage wt.)}$$

The fine root estimates from this equation are compared with those obtained above (Table A.2).

Table A.2 Comparison of fine root estimates with those from a published equation (Jackson and Chittenden 1981).

Treatment	This study	Jackson and Chittenden 1981
Control	741	1050
Single	1492	1488
3-Split	1873	1900
9-Split	1365	1396

The agreement is excellent for the fertilized trees but the published equation is apparently inappropriate for Control trees in this study.

## Appendix. 2

Statistical Analysis for Stem Diameters

Table A.3 Probability (p) of treatment differences in basal stem diameter according to single degree of freedom contrasts.

Date	Control vs fertilized	Single vs Split	3 vs 9 Split
	P		
January 1984	0.029	0.330	0.991
February	0.084	0.509	0.612
March	0.002	0.440	0.768
April	0.001	0.386	0.937
May	0.012	0.269	0.561
June	0.005	0.280	0.788
August	0.013	0.221	0.933
September	0.016	0.115	0.679

Table A.4 Probability (p) of treatment differences in stem diameter at 50 cm according to single degree of freedom contrasts.

Date	Control vs fertilized	Single vs Split	3 vs 9 Split
	P		
March 1984	0.061	0.384	0.839
April	0.049	0.352	0.781
May	0.070	0.288	0.894
June	0.036	0.199	0.726
August	0.094	0.245	0.822
September	0.074	0.173	0.943

## Appendix 3

Biomass and Fertilizer Recovery for Individual Trees

Table A.5 Final biomass and fertilizer recovery in relation to initial tree size.

Treatment	Initial D <sup>2</sup> H	Final biomass g/tree	Fertilizer recovery			Tree number
			Tree -----	Soil %	Total -----	
Single	28.4	11341	19.8	40.3	60.0	853
	26.3	10080	23.3	18.4	41.8	856
	23.1	10549	22.4	44.5	66.9	858
	28.1	11690	17.9	51.4	69.2	860
3-Split	34.3	13419	31.7	35.1	66.9	850
	34.7	12621	23.5	49.7	73.1	857
	16.3	10489	17.2	65.8	83.0	861
	31.0	10421	22.8	65.0	87.8	862
9-Split	18.6	10070	21.3	60.6	81.9	849
	21.1	10601	21.2	66.6	87.8	854
	36.7	11736	20.1	62.6	83.2	864
	17.9	8474	16.6	80.2	96.8	866
Autumn	18.9	6481	14.9	47.1	61.2	851
	19.5	7586	21.7	60.3	82.0	865
Spring	23.1	8626	23.1	75.7	98.8	846
	30.2	10444	25.5	69.0	94.5	847
Summer	29.9	9774	24.7	58.8	83.4	852
	17.6	6159	17.8	102.9	120.7	859

## Appendix 4

Initial Biomass of Radiata Pine at the Site in August 1983.

The above ground biomass of six trees was determined in August, 1983 at the age of 2 years. Height and diameter at the base were similar to the experimental trees. The trees were cut at ground level, transported to the laboratory, divided into components and oven dried at 65°C. The results are given in Table A.6.

Table A.6 Dry weight of six trees prior to the growing season under study, age 2-year-old, August, 1983.

Tree Component	Tree					
	A	B	C	D	E	F
	grams dry weight					
Foliage: 1-year	536	312	259	230	394	530
: older	96	86	64	69	93	99
Twigs : 1-year	168	88	53	94	129	132
: older	55	34	21	39	60	86
Stems : 1-year	43	56	35	46	65	53
: older	277	219	144	162	277	212
TOTAL	1175	795	576	740	1018	1112
Height (m)	1.75	1.73	1.49	1.43	1.71	1.49
Diameter (mm) at base	43.0	37.0	33.4	33.3	42.2	39.4

The relationships between  $D^2$ ,  $D^2H$  and the weight of tree components were investigated (Table A.7) in order to develop predictive equations (Table A.8).  $D^2$  was the preferred independent variable.



Table A.7 Correlation co-efficients between  $D^2$ ,  $D^2H$  and tree components for six trees sampled in August, 1983.

Predictive variable	Total	Components				All foliage
		1-year foliage	Older foliage	Twigs	Stem	
		r				
D <sup>2</sup> H	0.825	0.658	0.829	0.753	0.959	0.688
D <sup>2</sup>	0.908	0.774	0.889	0.856	0.943	0.799

Table A.8 Predictive equations of the form, Dry weight =  $a + b (D^2)$ , for six trees sampled in August, 1983.

Tree Components	a	b	$r^2$	significance
Total	-71.1	66.6	0.82	0.006
Foliage	7.1	32.2	0.64	0.028
Stem	-5.6	18.5	0.89	0.002
Twigs	-73.2	15.9	0.73	0.015
1-year twigs	-54.5	11.3	0.80	0.008
older twigs	-18.7	4.6	0.42	0.082
1-year stem	25.7	1.6	0.25	0.159
older stem	-31.4	16.8	0.94	0.001
1-year twigs and stem	-28.8	12.9	0.92	0.005
older twigs and stem	-50.0	21.5	0.95	0.000

## Appendix 5

Use of Co-variance Analysis

Although an effort was made to select similar trees (Chapter 2), there was still a difference between treatment means as shown by initial  $D^2H$  (Tables 3.6 and 3.7). In retrospect, it may have been wiser not to use simple random sampling in allocating trees to treatments.

Because initial tree size was considered an important factor, a suitable co-variate was required to remove this effect. Four co-variates were investigated:

- (i) (initial diameter at stem base)<sup>2</sup>,
- (ii) (initial diameter at 50 cm up stem)<sup>2</sup>,
- (iii) (initial diameter at base)<sup>2</sup> x height.
- (iv) older foliage biomass (Tables 3.6 and 3.7).

Effective use of co-variance usually requires a linear relationship between the calibrating variate and the measured variate (Cellier and Correll 1984). The correlation between the four possible co-variates and final tree biomass was:

Co-variate	Correlation co-efficient (r)
$D^2_{base}$	0.624
$D^2_{50\ cm}$	0.622
$D^2_{base}H$	0.670
Older foliage	0.688

A general assumption in co-variance analysis is that co-variates are not effected by treatment. This is clearly satisfied for initial stem dimensions, but what about older foliage, measured at the end of the experiment? This component consisted of fascicles formed in the two years following planting and prior to any fertilizer applications. A few lower stem needles were senescing, but actual needle fall was minimal. These fascicles increased in weight during winter 1983, but this was unaffected by treatment (Table 3.18). An estimate of needle weight in the older foliage at the final harvest showed a 20-30% decrease in weight since the previous November. This decrease was not apparently affected by fertilizer treatment.

Older foliage was probably a valid co-variate, but the analyses of co-variance in this study generally use initial  $D^2H$ . These two co-variates give different adjusted means, e.g. for 1-year foliage at the final harvest (Table A.9).

Table A.9 Comparison of two co-variates for adjusted 1-year foliage at final harvest.

	Control	Single	3-Split	9-Split
	g			
Actual	1571	2002	2196	1696
With initial D <sup>2</sup> H	1568	2000	2104	1793
With old foliage	1589	1948	1988	1940

Clearly the older foliage co-variate adjusted the means further, e.g. 9-Split was increased more and 3-Split decreased more.

In using analysis of co-variance for nitrogen contents and fertilizer recovery (Chapter 6), the use of older foliage as a co-variate may have had more biological appeal. Foliage was the main sink for fertilizer and constituted the largest nitrogen pool within the tree. However, to maintain comparability initial D<sup>2</sup>H was used.

## Appendix 6

Single Degree of Freedom Contrasts From ANCOVA With Initial D<sup>2</sup>H  
For Biomass Components.

Tree component	Contrast		
	Control vs fertilized	Single vs Split	3-Split vs 9-Split
	p		
TOTAL	0.000	0.882	0.275
Above ground	0.002	0.994	0.141
Below ground	0.000	0.798	0.597
1-year foliage	0.005	0.683	0.062
Stem	0.029	0.351	0.735
Older stem wood	0.026	0.543	0.772
1-year wood	0.030	0.681	0.358
Rootstock	0.024	0.581	0.054
Coarse roots	0.015	0.885	0.747
Fine roots	0.001	0.272	0.056

p: probability of differences between specified contrasts.

## Appendix 7

Needle Weights and Lengths

Table A.11 Needle weight in the upper crown as affected by fertilizer, main treatments, adjusted means.

Date		Control	Single	3-Split	9-Split	(SE)	P
		----- mg per needle -----					
November	15	7	8	9	8	(0.8)	0.473
November	28	9	10	10	11	(0.9)	0.510
December	13	10	11	12	14	(1.3)	0.424
December	28	14	17	16	17	(1.6)	0.421
January	16	19	23	22	25	(1.8)	0.177
January	24	21	25	23	28	(1.9)	0.162
February	7	23	28	27	30	(1.7)	0.069
February	21	27	34	30	35	(1.6)	0.016
March	6	31	35	34	39	(1.5)	0.015
March	27	33	34	32	42	(1.5)	0.004
April	7	33	39	35	41	(2.2)	0.087
May	5	35	39	36	42	(2.0)	0.108
June	20	39	45	41	47	(1.9)	0.037
July	5	39	44	40	47	(2.7)	0.189
August	21	40	40	41	47	(2.2)	0.076
September	28	42	47	42	50	(2.2)	0.083

(SE): standard error.

p : probability of treatment differences according to ANCOVA.

Table A.12 Needle weight in the middle crown as affected by fertilizer, main treatments, adjusted means.

Date		Control	Single	3-Split	9-Split	(SE)	P
		----- mg per needle -----					
November	15	4	4	4	5	(0.5)	0.545
November	28	6	6	7	7	(0.7)	0.545
December	13	7	8	9	9	(0.9)	0.581
December	28	9	10	11	12	(1.0)	0.381
January	16	13	16	15	17	(1.3)	0.158
January	24	14	16	17	19	(1.4)	0.080
February	7	16	21	20	22	(1.2)	0.016
February	21	21	25	25	27	(1.6)	0.168
March	6	24	27	26	30	(1.6)	0.166
March	27	23	28	30	31	(1.8)	0.042
April	7	26	29	28	33	(1.6)	0.075
May	5	25	29	29	33	(1.9)	0.093
June	20	32	36	32	38	(2.3)	0.243
July	5	34	36	32	41	(1.7)	0.023
August	21	30	35	32	38	(2.9)	0.320
September	28	31	36	34	41	(2.5)	0.098

(SE): standard error.

p : probability of treatment differences according to ANCOVA.

Table A.13 Needle length in the middle crown as affected by fertilizer, main treatments, adjusted means.

Date		Control	Single	3-Split	9-Split	(SE)	P
		----- mm per needle -----					
November	15	33	32	36	37	(1.8)	0.185
November	28	41	43	46	46	(2.6)	0.417
December	13	51	53	58	58	(2.4)	0.157
December	28	62	66	70	71	(3.2)	0.234
January	16	79	87	87	90	(3.0)	0.121
January	24	86	92	97	96	(2.8)	0.055
February	7	96	105	105	106	(2.5)	0.040
February	21	113	120	122	119	(3.5)	0.355
March	6	126	128	127	132	(3.2)	0.626
Mar-Sept*		132	134	130	139	(2.6)	0.120

(SE): standard error.

p : probability of treatment differences according to ANCOVA.

\* : data for 7 dates (Mar. 27 - Sept. 28) combined.

Table A.14 Needle length in the upper crown as affected by fertilizer, main treatments, adjusted means.

Date		Control	Single	3-Split	9-Split	(SE)	P
		----- mm per needle -----					
November	15	45	45	50	49	(2.7)	0.541
November	28	56	57	59	61	(3.2)	0.705
December	13	65	68	70	73	(3.9)	0.533
December	28	77	82	83	84	(4.1)	0.616
January	16	97	99	103	106	(3.2)	0.235
January	24	105	109	106	114	(3.6)	0.401
February	7	112	121	117	120	(3.1)	0.303
February	24	127	135	125	131	(3.4)	0.237
March	6	136	141	134	143	(2.7)	0.117
Mar-Sept*		144	147	138	149	(3.1)	0.135

(SE): standard error.

p : probability of treatment differences according to ANCOVA.

\* : combined data for 7 dates (March 27 - September 28).

## Appendix B

Results for IUFRO Standards

The 1984 and 1985 IUFRO interlaboratory comparison samples were analysed for a number of elements using XRF.

Table A.15 Foliar concentrations of P, K, Ca, and Mg in IUFRO samples.

		Thomas	International value	
		Lab.51	Mean (s.d.)	Median (mad.)
		-----	% oven dry weight	-----
84/1 radiata pine foliage	P -	0.144	0.143 (0.008)	0.140 (0.002)
	K -	1.04	0.91 (0.08)	0.90 (0.04)
	Ca-	0.22	0.22 (0.03)	0.22 (0.01)
	Mg-	0.13	0.12 (0.01)	0.12 (0.01)
84/2 <i>Eucalyptus nitens</i> foliar	P -	0.130	0.133 (0.009)	0.132 (0.005)
	K -	0.88	0.76 (0.09)	0.78 (0.04)
	Ca-	0.54	0.52 (0.05)	0.53 (0.05)
	Mg-	0.11	0.10 (0.01)	0.10 (0.01)
85/1 tulip tree foliage	P -	0.132	0.128 (0.007)	0.130 (0.005)
	K -	1.53	1.45 (0.13)	1.46 (0.06)
	Ca-	1.29	1.23 (0.18)	1.22 (0.06)
	Mg-	0.42	0.34 (0.03)	0.33 (0.01)
85/2 douglas fir foliage	P -	0.100	0.105 (0.006)	0.105 (0.005)
	K -	0.61	0.58 (0.03)	0.58 (0.03)
	Ca-	0.39	0.38 (0.03)	0.38 (0.03)
	Mg-	0.11	0.11 (0.01)	0.11 (0.01)
85/3 radiata pine foliage	P -	0.128	0.124 (0.008)	0.126 (0.005)
	K -	0.63	0.53 (0.05)	0.54 (0.03)
	Ca-	0.19	0.18 (0.02)	0.18 (0.01)
	Mg-	0.09	0.10 (0.01)	0.09 (0.01)
85/4 radiata pine wood	P -	0.012	0.026 (0.03)	0.013 (0.003)
	K -	0.14	0.12 (0.06)	0.11 (0.01)
	Ca-	0.05	0.06 (0.06)	0.05 (0)
	Mg-	0.02	0.04 (0.04)	0.03 (0.01)
85/5 radiata pine bark	P -	0.036	0.038 (0.03)	0.039 (0.001)
	K -	0.42	0.34 (0.03)	0.34 (0.02)
	Ca-	0.21	0.20 (0.03)	0.20 (0.01)
	Mg-	0.06	0.09 (0.11)	0.07 (0.01)



85/6 radiata pine P -	0.111	0.105 (0.020)	0.110 (0.004)
litter K -	0.47	0.40 (0.05)	0.40 (0.02)
Ca-	0.40	0.37 (0.06)	0.37 (0.02)
Mg-	0.07	0.09 (0.12)	0.07 (0.01)

---

International values are from Will (1986).

The majority of laboratories have probably analysed the samples using chemical methods. The X-ray results are generally higher, particularly so for potassium. This could be due to losses which may have occurred during chemical preparation (Norrish and Hutton 1977). However, it was noted earlier that the XRF results are only as good as the calibration. Ideally, a further set of standards should have been obtained to check the calibration used in this study.

For the purpose of treatment comparisons in Chapters 4 and 7, the discrepancy is not important. In comparing the absolute values with published standards, it should be noted that:

- (1) the agreement for P, Ca, and Mg with international values is good for the range of concentrations in this study.
- (2) the potassium levels, even if "corrected" to agree with the IUFRO means, are still satisfactory for the growth of radiata pine.

The potassium levels presented in this study should be used with caution, if total K pools are calculated. However, there is a good correlation between the X-ray results and international means, so a correction could be made:

$$\begin{aligned} \text{IUFRO (\% K)} &= 0.9557 (\text{XRF, \% K}) - 0.0481 & r^2 &= 0.997 \\ &(\text{SE } 0.0196) & & (\text{SE } 0.0161) \end{aligned}$$

If the regression is forced through the origin the equation becomes:

$$\text{IUFRO (\% K)} = 0.9045 (\text{XRF, \% K}) \quad r^2 = 0.998$$

The other element investigated in this study was sulphur. Only a few of the participating laboratories in the IUFRO comparison report sulphur values. These are compared with the X-ray results obtained in this study (Table A.16). The agreement was generally satisfactory and enables the calculations of sulphur nitrogen ratios to be performed with some confidence. Of course, if the laboratories were actually named, then the XRF sulphur values could be compared directly with the results of Kelly and Lambert (1972).

Table A.16 Sulphur results for IUFRD samples.

IUFRD sample	Thomas Lab.51	International	Number of laboratories
		mean ----- % oven dry weight -----	
84/1	0.116	0.101	7
84/2	0.148	0.143	7
85/1	0.267	0.244	10
85/2	0.086	0.085	10
85/3	0.113	0.103	10
85/4	0.012	0.025	10
85/5	0.037	0.043	10
85/6	0.078	0.070	10

#### Conclusion

The use of XRF enables a large number of elements to be analysed quickly and easily, once problems of pellet preparation have been overcome. Further comparisons of X-ray and chemical methods are required before results of the latter can be applied to current standards. However, the main requirement is for workers to detail their analytical methods and to include their result for an international standard in all published work.

## Appendix 9

Sulphur : Nitrogen Interaction

Table A.17 Sulphur and nitrogen status of Single treatment trees.

Date	Total N ----- % -----	Total S -----	S:N gram atom basis	SO <sub>4</sub> <sup>==</sup> (ppm)	µg S/needle
2 May.	1.663	0.113	0.030	12	24
22 Aug. (F)	1.705	0.136	0.035	190	31
18 Oct.	1.975	0.139	0.031	34	36
1 Nov.	2.003	0.146	0.032	85	36
15 Nov.	1.879	0.121	0.028	0	30
Upper crown					
28 Nov.	1.995	0.117	0.026	0	30
24 Jan.	1.526	0.093	0.027	0	24
21 Feb.	1.462	0.096	0.029	0	32
27 Mar.	1.501	0.101	0.029	0	37
20 June	1.370	0.106	0.034	120	48
28 Sept.	1.467	0.117	0.035	163	55
Middle crown					
28 Nov.	1.773	0.101	0.025	0	6
24 Jan.	1.539	0.096	0.027	0	15
21 Feb.	1.484	0.093	0.027	0	23
27 Mar.	1.472	0.095	0.028	0	27
20 June	1.331	0.098	0.032	66	35
28 Sept.	1.375	0.096	0.030	16	35
Final biomass					
9 Oct.	1.519	0.105	0.030		
	1.423	0.107	0.033		
	1.419	0.100	0.031		
	1.592	0.116	0.032		
	-----	-----	-----		
	1.488	0.107	0.031		

(F): fertilizer applied.

Table A.18 Sulphur and nitrogen status of Control trees.

Date	Total N ----- % -----	Total S	S:N	SO <sub>4</sub> -- (ppm)	µg S/needle
2 May	1.561	0.115	0.032	78	23
22 Aug.	1.590	0.144	0.039	349	32
18 Oct.	1.801	0.167	0.040	434	41
1 Nov.	1.850	0.174	0.041	470	45
15 Nov.	1.663	0.151	0.040	368	36
Upper crown					
28 Nov.	1.918	0.120	0.027	0	11
24 Jan.	1.467	0.106	0.031	53	23
21 Feb.	1.502	0.116	0.034	130	31
27 Mar.	1.591	0.123	0.034	152	41
20 June	1.530	0.137	0.039	320	53
28 Sept	1.660	0.165	0.043	510	70
Middle crown					
28 Nov.	1.684	0.116	0.030	4	7
24 Jan.	1.401	0.104	0.032	78	14
21 Feb.	1.483	0.108	0.032	62	23
27 Mar.	1.455	0.102	0.031	21	19
20 June	1.444	0.118	0.036	189	39
28 Sept	1.478	0.130	0.038	285	41
Final biomass					
9 Oct.	1.698	0.158	0.041		
	1.376	0.131	0.042		
	1.565	0.131	0.137		
	1.776	0.143	0.035		
	-----	-----	-----		
	1.604	0.141	0.039		

Table A.19 Data for 3-Split treatment trees.

Date	Total N ----- % -----	Total S ----- % -----	S:N	SO <sub>4</sub> -- (ppm)	µg S/needle
2 May (F)	1.513	0.123	0.035	191	23
27 June	1.709	0.141	0.036	237	30
22 Aug.	1.810	0.153	0.037	298	34
18 Oct.	2.075	0.143	0.030	6	35
15 Nov.	1.893	0.141	0.032	111	35
Upper crown					
28 Nov.	2.027	0.123	0.026	0	13
21 Feb.	1.468	0.096	0.028	0	29
20 June	1.406	0.118	0.037	215	48
Final					
biomass: tree					
9 Oct.	850 1.331	0.124	0.041		
	857 1.600	0.132	0.035		
	861 1.416	0.110	0.034		
	862 1.468	0.120	0.036		
	-----	-----	-----		
	1.454	0.121	0.036		

(F): fertilizer applied.

Table A.20 Sulphur and nitrogen status of 9-Split trees.

Date	Total N ----- % -----	Total S ----- % -----	S:N	SO <sub>4</sub> -- (ppm)	µg S/needle
2 May (F)	1.585	0.118	0.032	92	29
27 June (F)	1.868	0.162	0.038	338	37
18 Oct. (F)	2.464	0.167	0.030	0	43
15 Nov. (F)	2.079	0.152	0.032	93	36
Final biomass					
9 Oct.	1.679	0.138	0.036		
	1.552	0.137	0.038		
	1.699	0.135	0.035		
	1.561	0.108	0.030		
	-----	-----	-----		
	1.623	0.129	0.035		

(F): fertilizer applied.

## Appendix 10

Nitrate Concentrations in Soil Water Samples

Table A.21 Concentration of nitrate in soil solution.

Treatment	Depth (cm)	Date			
		February 28	March 13	July 5	July 8
		----- ppm NO <sub>3</sub> <sup>-</sup> -----			
Control	20	-	2.41	-	0.48
	40	-	-	-	1.26
	80	-	1.68	2.10	1.12
Single	20	4.76	1.40	-	-
	40	-	0.10	-	0.60
	80	15.40	1.68	1.68	0.78
3-Split	20	4.20	0.20	1.20	0.60
	40	-	-	-	-
	80	23.80	-	-	0.76
9-Split	20	2.70	0.42	0.98	0.56
	40	13.16	9.73	-	6.16
	80	0.84	4.90	5.88	0.95
Autumn	20	2.80	1.75	2.52	1.57
	40	2.80	0.91	1.47	0.70
	80	8.19	0.21	-	2.10
Spring	20	-	0.10	-	0.56
	40	0.66	-	-	1.15
	80	-	-	-	-
Summer	20	-	1.26	-	2.10
	40	0.70	3.78	-	0.70
	80	4.06	3.22	-	2.80

Mean of two lysimeters when available.

-: no sample available.

## Appendix 11

Nitrogen Results from Periodic Soil Samples

Table A.22 Nitrogen concentrations at 0-10 cm depth.

Plot	Date				
	August 18	October 18	December 12	February 7	June 20
	----- % air dry weight -----				
853	0.1731	0.0596	0.1004	0.0826	0.0644
856	0.0702	0.0559	0.1139	0.0453	0.0836
858	0.0529	0.1182	0.0605	0.0636	0.0699
860	0.1035	0.1192	0.0973	0.0813	0.0696
Mean	0.0999	0.0875	0.0930	0.0682	0.0719

Table A.23 Nitrogen concentration at 10-30 cm depth.

Plot	Date				
	August 18	October 18	December 12	February 7	June 20
	----- % air dry weight -----				
853	0.0499	0.0133	0.0188	0.0146	0.0144
856	0.0199	0.0162	0.0166	0.0187	0.0136
858	0.0134	0.0174	0.0166	0.0166	0.0135
860	0.0259	0.0189	0.0154	0.0209	0.0143
Mean	0.0253	0.0164	0.0168	0.0177	0.0139

Table A.24 Atom % N-15 for 0-10 and 10-30 cm depths.

Plot	(cm)	Date				
		August 18	October 18	December 12	February 7	June 20
		----- atom % N-15 -----				
853	0-10	0.357	0.526	0.456	(0.639)	0.460
	10-30	0.358	0.548	0.415	0.431	0.431
856	0-10	0.355	0.486	0.370	0.423	0.379
	10-30	0.357	0.443	0.377	0.402	0.384
858	0-10	0.357	0.425	0.395	0.540	0.464
	10-30	0.356	0.403	0.379	0.459	0.425
860	0-10	0.358	0.529	0.464	0.383	0.472
	10-30	0.360	0.593	0.486	0.427	0.445

Table A.25 Recovery of fertilizer nitrogen at 0-10 and 10-30 cm depths.

Plot	(cm)	Date			
		October 18	December 12	February 7	June 20
		%			
853	0-10	35.6	36.8	(86.3)	24.6
	10-30	24.4	10.3	10.3	10.1
856	0-10	36.5	7.5	15.1	9.3
	10-30	14.1	3.2	8.4	3.6
858	0-10	31.9	9.1	46.2	29.7
	10-30	7.8	3.5	16.8	9.0
860	0-10	64.9	32.9	6.7	25.3
	10-30	43.7	19.4	14.2	12.2



## Appendix 12

Estimate of Initial Nitrogen Content of Control Trees

The estimate of annual nitrogen uptake required an initial nitrogen content. This was estimated for Control trees in August 1983 (early spring). Their biomass was estimated using regressions developed from the August 1983 fellings (Appendix 4). The predictive equations used were for foliage, 1-year twigs and stem combined, and older twigs and stem combined (Table A.8). The results are given in Table A.26.

Table A.26 Dry weight estimates of Control trees in August 1983.

Tree	D <sup>2</sup>	Foliage	1-year twigs	Older twigs
			and stem	and stem
		----- dry weight grams -----		
848	19.80	644	227	375
855	18.83	613	215	355
863	16.16	527	180	297
867	18.15	591	206	340

The dry weight of old foliage at the final harvest was considered to be an approximation of initial foliage biomass (Appendix 5). The treatment mean in Table A.26 is in reasonable agreement with this measured value, i.e.

Tree	Old foliage (Table 3.6)
	(g)
848	500
855	596
863	541
867	608

---  
561 vs 594 from Table A.26

The nitrogen content was obtained by using appropriate %N values. The actual foliar nitrogen concentrations for August 22 were used. For 1-year twigs and stem the final biomass %N for new twigs was used. For older twigs and stem the final biomass %N for older twigs was used. The mean nitrogen content above ground for the Control trees in August 1983 was 11.7 g N (Table A.27).

Table A.27 Estimated nitrogen content of Control trees in August 1983.

Tree	Foliage	1-year twigs	Older twigs	Total
		and stem	and stem	
		g		
848	9.29	1.09	1.17	11.55
855	10.49	0.91	0.96	10.57
863	8.12	1.03	1.27	10.42
867	9.84	1.28	1.27	12.39

## Appendix 13

Estimation of Foliage Biomass on Surrounding Trees

An estimate of 1-year foliage biomass for surrounding trees was required to assess fertilizer uptake by surrounding trees (Appendix 14). Predictive equations are available in the literature for radiata pine (e.g. Madgwick 1983b), however, Baker *et al.* (1984:) consider that it is always better to sample within the stand of interest. Accordingly regression equations were developed from the final biomass data. The independent variables  $D$ ,  $D^2$ ,  $D^2H$  and  $D/H$  were investigated ( $D$  and  $H$  being diameter at stem base and height at the final biomass).  $D^2H$  gave a better fit than  $D$  or  $D^2$ . Incorporation of  $D/H$  in addition to  $D^2H$  did not improve the equations. Simple linear regressions with logarithmic transformation of independent and dependent variables were developed:

$$\text{Ln (weight tree component)} = a_0 + a_1 \text{Ln } (D^2H) \quad (1)$$

Data from all 22 trees was used but care was taken to check whether the regressions were homogenous across the three rates of nitrogen used: 0,30 and 90 g N (c.f. Snowdon 1985).

A single regression was found to be applicable across all treatments for 1-year foliage:

$$\text{Ln (1-year foliage g)} = 2.992 + 0.830 \text{Ln } (D^2H) \quad (2)$$

$r^2=0.64, n=22$

The applicability of a single equation contrasts with Snowdon (1985) and Grier *et al.* (1984) who found separate equations for fertilized and unfertilized trees were required. Whether the allometric relationship is changed (which these authors are essentially arguing) will depend upon the response to fertilizer. So for example Grier *et al.* (1984) altered the trees allometry for 1-year foliage but not for total foliage in 23-year-old douglas fir. Given the large response below ground in this study a single predictive equation was not surprisingly found to be inappropriate for this component.

The equation to predict 1-year foliage was refined by incorporating the data from six 2-year-old trees (Appendix 4). This was initially incorporated as another treatment to check for homogeneity across ages (c.f. Crow 1983). A single regression equation was still valid:

$$\text{Ln (1-year foliage)} = 3.794 + 0.682 \text{Ln } (D^2H) \quad (3)$$

$r^2=0.95, n=28$

This equation was used to estimate the foliage biomass of surrounding trees. In converting back to arithmetic units a bias is incurred

(Baskerville 1972). Consequently estimates were corrected for with the factor used by Madgwick (1983b):

exponential (0.5 error mean square)

Biomass estimates from equation (3) were therefore multiplied by 1.013.

## Appendix 14

Estimate of Fertilizer Nitrogen Utilised by Surrounding Trees

An estimate of N-15 utilisation was made for trees surrounding the four Single treatment plots. Samples of the 1-year foliage on trees within one metre of the plot edge were taken on October 19, 1984. The height and diameter at the base of the sampled trees were measured so that foliage biomass could be predicted from regression equations.

The foliage samples were bulked by plot and analysed for total nitrogen and N-15 as in Chapter 5. The biomass of 1-year foliage was estimated from equation (3) in Appendix 13.

$$\ln (1\text{-year foliage g}) = 3.794 + 0.682 \ln (D^2H) \quad r^2=0.95, n=28$$

The estimated foliage biomass and nitrogen results are given in Table A.28.

Table A.28 Parameters of surrounding trees to estimate fertilizer uptake.

Plot	Adjacent trees				Atom % N-15
	1-year foliage (grams)		% N		
853	1989	1907		1.613	0.384
856	593	2055	1743	1.771	0.375
858	1120	2157	2048	1.740	0.403
860	841	1857	871	1.814	0.365

The uptake of fertilizer into the 1-year foliage was calculated as in Chapter 6, p.112. The uptake for the whole tree was estimated from the proportion of fertilizer that occurred in the 1-year foliage of Single treatment trees (Table 6.10). This was 47.4% which compares with 41% used by Mead (1971). The recovery of applied nitrogen in surrounding trees is given in Table A.29.

Table A.29 Recovery of applied fertilizer in trees surrounding Single treatment plots.

Plot	Surrounding trees			Total
	----- % of applied -----			
853	0.74	0.48		1.22
856	0.17	0.59	0.51	1.27
858	0.78	1.52	1.45	3.75
860	0.10	0.23	0.10	0.43

## Appendix 15

## Statistical Terminology

The standard error (SE) given in most tables is the standard error of the pooled treatment mean, given as:

$$\sqrt{\frac{s^2}{r}} \quad (\text{Steel and Torrie p.143 1981})$$

where  $s^2$  = error mean square from ANOVA or ANCOVA  
 $r$  = replication within the treatment.

The probability (p) of treatment differences according to ANOVA or ANCOVA is a significance level e.g.  $p = 0.050$  is the conventional 5% significance level (\*).